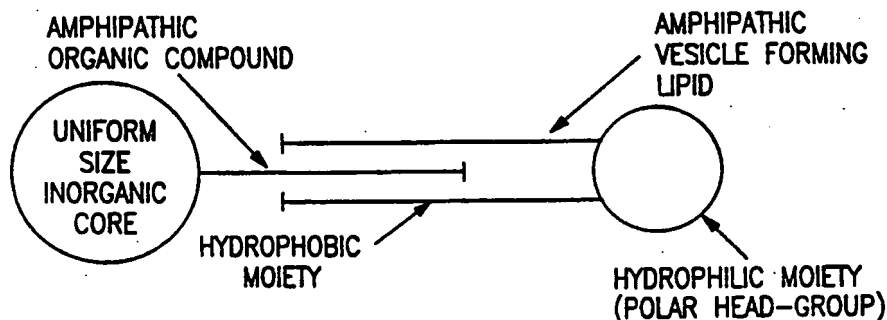




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : H01F 1/06, G01N 33/543 C12N 11/00, G01N 33/58 C01G 1/02, A61K 9/127, 49/00 C07C 69/58, C08G 65/26	A1	(11) International Publication Number: WO 93/26019 (43) International Publication Date: 23 December 1993 (23.12.93)												
(21) International Application Number: PCT/US93/05595 (22) International Filing Date: 8 June 1993 (08.06.93) (30) Priority data: <table border="0"> <tr> <td>894,260</td> <td>8 June 1992 (08.06.92)</td> <td>US</td> </tr> <tr> <td>911,962</td> <td>10 July 1992 (10.07.92)</td> <td>US</td> </tr> <tr> <td>958,646</td> <td>7 October 1992 (07.10.92)</td> <td>US</td> </tr> <tr> <td>057,687</td> <td>5 May 1993 (05.05.93)</td> <td>US</td> </tr> </table> (71) Applicant: MOLECULAR BIOQUEST, INC. [US/US]; 8 Commerce Drive, Atkinson, NH 03811 (US). (72) Inventors: CHAGNON, Mark, S. ; 10 Valleyview Drive, Pelham, NH 03076 (US). CARTER, Michelle, J. ; 270 Island Pond Road, Derry, NH 03038 (US). FERRIS, John, R. ; 31 Union Street, Newburyport, MA 01950 (US). GRAY, Maria, A. ; 1 Mirra Avenue, Derry, NH 03038 (US). HAMILTON, Tracy, J. ; 14 Eagle Drive, Salem, NH 03079 (US). RUDD, Edwin, A. ; 52 Brookdale Road, Salem, NH 03079 (US).		894,260	8 June 1992 (08.06.92)	US	911,962	10 July 1992 (10.07.92)	US	958,646	7 October 1992 (07.10.92)	US	057,687	5 May 1993 (05.05.93)	US	(74) Agent: SOLOWAY, Norman, P.; Hayes, Soloway, Hennessey & Hage, 175 Canal St., Manchester, NH 03101 (US). (81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
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(54) Title: PREPARATION OF CONTROLLED SIZE INORGANIC PARTICLES FOR USE IN SEPARATIONS, AS MAGNETIC MOLECULAR SWITCHES, AND AS INORGANIC LIPOSOMES FOR MEDICAL APPLICATIONS

**(57) Abstract**

Inorganic oxides of substantially uniform particle size distribution are prepared by contacting aqueous solutions of an inorganic salt and an inorganic base across a porous membrane (14) wherein the membrane contains a plurality of pores which allows for precipitation of a substantially mono-dispersed size inorganic oxide particles on one side of the membrane and precipitation of a salt of the corresponding base on a second side of the membrane (Fig. 1). The particles so prepared can be coated with an organo-metallic polymer having attached thereto an organic functionality to which a variety of organic and/or biological molecules can be coupled. Particles so coupled may be used for in vitro or in vivo systems involving separations steps or the directed movement of coupled molecules to particular sites, including immunological assays, other biological assays, biochemical or enzymatic reactions, affinity chromatographic purification, cell sorting and diagnostic and therapeutic uses. In a further embodiment, described herein are liposome compositions which comprise the substantially uniform size inorganic core coated with an amphiphatic organic compound and further coated with a second amphiphatic vesicle forming lipid (Fig. 2). Also disclosed are novel phenyl lipid compounds which serve as the vesicle forming lipid (Fig. 3). When the magnetic particles are electromagnetic wave-absorbing surface modified particles (Fig. 4), such particles provide for the preparation of liposome compositions which offer a method for the treatment of cancer, as well as infectious diseases.

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1 PREPARATION OF CONTROLLED SIZE INORGANIC PARTICLES
2 FOR USE IN SEPARATIONS, AS MAGNETIC MOLECULAR SWITCHES,
3 AND AS INORGANIC LIPOSOMES FOR MEDICAL APPLICATIONS

5 Field of the Invention

6 This invention relates to a method for producing
7 inorganic oxides of substantially uniform particle size
8 distribution, coating said particles with various
9 functional moieties, and clustering said moieties together
10 via controllably degradable chemical, complex, or ionic
11 bonds. More particularly, this invention relates to a
12 method of producing magnetic inorganic oxide particles of
13 substantially uniform size, or organic coated particle
14 beads, linking the particle or particle bead together to
15 form a large aggregate cluster with different chemical,
16 physical, or magnetic properties than the unit particle or
17 bead, and controllably and predictably revising the
18 cluster back to unit bead or particle size and vice versa.

19 The substantially uniform size inorganic oxides also
20 allow for the preparation of novel inorganic core liposome
21 compositions for in vivo and in vitro medical
22 applications.

23 Background of the Invention

24 Separations of all types are routinely done by the
25 exploitation of physical and chemical differences in the
26 various species to be separated. Size exclusion, boiling
27 point, and chemical affinity are techniques that have been
28 used for separations of particles, chemical species, and
29 biological moieties for hundreds of years. More recently,
30 the use of magnetism has been used as a tool for
31 separation of various species material from one another.
32 By the early 1960's, the first stable magnetic fluid
33 colloid had been described. Later research led to the
34 development of a separations device based on magnetic
35 density gradients in magnetic fluid columns. By 1979,
36 magnetic particles coated with appropriate functional

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1 chemical groups for affinity chromatography separations
2 were reported. The first commercial application of
3 magnetic separations was described by Chagnon et al in
4 U.S. Patent No. 4,628,037. The Chagnon patent describes
5 the use of amine terminated silane coupled magnetic
6 particles for immunodiagnostic applications. The
7 materials described in the Chagnon et al patent are now
8 used commercially in medical diagnostic kits.

9 Magnetic separations have not been exclusively applied
10 to in vitro applications. The use of magnetic separations
11 for in vivo applications is becoming increasingly more
12 accepted and important as a therapeutic and diagnostic
13 tool. By the early 1980's, published reports described
14 the magnetic targeting and isolation of chemotherapeutic
15 drugs into rat-tail sarcoma. Widder (U.S. Patent Nos.
16 4,849,210; 4,247,406; and 4,230,685) describe the use of
17 magnetic albumin spheres for ultrasound contrast media and
18 magnetic drug targeting. Schroeder (U.S. Patent No.
19 4,501,726) reports a method of preparing magnetic starch
20 beads for use in MRI imaging for the separation of T_1/T_2
21 relaxation signals.

22 In all of this previous work, the use of magnetic
23 separations has been done on magnetic particles of varying
24 particle size distribution. The magnetic particle is
25 coated with an organic compound, and used either as a
26 signal (e.g., MRI), targeting agent (e.g. in drug
27 delivery) or for separation in a magnetic field (e.g. in
28 vitro separations). However, an advantage in enhanced
29 separations, for example, could be achieved if the
30 magnetic particle could alter its size, shape or magnetic
31 properties while in use in a controlled fashion.

32 Various methods have been reported for preparing
33 inorganic or inorganic oxide particles of some degree of
34 particle size control:

35 U.S. Patent 5,071,076 describes a method for producing
36 magnetic microparticles from metallocenes. The method

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1 involves combining an aqueous slurry of the metallocene
2 and an aqueous slurry of a metal hydroxide and milling the
3 slurries together.

4 U.S. Patent 4,987,012 describes a process for
5 preparing spherical particles of hydroxide having a
6 particle diameter from 0.1 to 10. μ m by adding a
7 corresponding metal alkoxide to a dispersion of a water-
8 alcohol system having dispersed therein a metal oxide or
9 hydroxide as a seed, under alkaline conditions and
10 allowing a decomposition product from said metal alkoxide
11 to attach onto said seed to effect particle growth of the
12 seed. The improvement reported comprises maintaining said
13 dispersion at a substantially constant pH within the range
14 between 10 and 13 during the addition of the metal
15 alkoxide to said dispersion and the subsequent particle
16 growth of the seed, thereby to prepare mono-dispersed
17 particles substantially free from particle aggregation
18 having a sharp particle size distribution of a standard
19 deviation of not greater than 0.5.

20 U.S. Patent 4,985,273 describes a method of producing
21 fine inorganic particles. The method comprises the steps
22 of reacting an inorganic fine particle on the entire
23 surface thereof with a silane type surface active agent
24 containing a straight hydrocarbon chain and a functional
25 group to form a monomolecular film on the entire surface
26 of said inorganic fine particle, thereafter making the
27 inorganic fine particles covered with the monomolecular
28 film in a predetermined density on a substrate, and
29 thereafter subjecting the monomolecular film to physical
30 or chemical treatment to allow the functional groups to be
31 chemically bonded to each other.

32 U.S. Patent 4,945,049, reports on a method for
33 preparing magnetic powder comprising homogeneous and fine
34 particles using an alkali-producing enzyme. Particles
35 having a particle size ranging from 50 to 500 nm's were
36 reported.

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1 U.S. Patent 4,702,775 describes the control of
2 particle size in the preparation of magnetite pigments.
3 The mean particle size was brought to a value within the
4 range of 0.06 to 0.5 μm by means of a residence stage
5 between the precipitation stage and the oxidation stage.
6 Various other disclosures describe the preparation of
7 microporous membranes, primarily for a filtration purpose,
8 which limit the passage of selected size molecules within
9 a particular liquid medium. For example, U.S. Patent
10 4,943,374 concerns the use of a microporous membrane
11 constructed of a polyether sulfone and hydrophilization
12 agent having a pore size which is within the range of 0.1
13 and 1.2 microns for the filtration of beer. U.S. Patent
14 4,954,381 describes the preparation of porous substrates
15 having well defined morphology. U.S. Patent 4,964,992
16 describes a membrane filter having predetermined
17 controlled porosity and to the method for making such a
18 membrane filter. U.S. Patent 5,057,226 describes a method
19 of removing a constituent of a biological fluid including
20 a blood component, said method including flowing the
21 biological fluid past one side of a first semipermeable
22 membrane, flowing solution containing a first
23 precipitation agent past a second side of the membrane so
24 as to cause transfer of the precipitation agent through
25 the membrane to the biological fluid so as to improve
26 precipitation characteristics of the fluid; and
27 precipitating the constituent.

28 What emerges from the above, therefore, is the lack of
29 a convenient method to control inorganic oxide particle
30 size, such that particle size control can then be further
31 utilized to manufacture novel aggregate particle clusters
32 with unique chemical or physical-chemical properties.

33 Accordingly, it is an object of this invention to
34 provide a method for producing inorganic oxides of
35 substantially uniform particle size, coating said
36 particles with various functional moieties, and clustering

1 said moieties together via controllably degradable
2 chemical, complex or ionic bonds.

3 It is also an object of this invention to provide a
4 method of producing magnetic particle or organic coated
5 particle beads, linking said particle or particle beads
6 together to form a large aggregate cluster with different
7 chemical, physical, or magnetic properties than the unit
8 particle or bead from which it is derived, and
9 controllably and predictably revising the cluster back to
10 unit bead or particle, and vice versa.

11 It is also a further object of this invention to
12 provide a method of producing unit magnetic crystals of
13 small, substantially uniform particle size for use in
14 preparing magnetic-molecular switches and apply such to
15 several in vitro and in vivo medical and biological
16 applications.

17 Nomenclature

18 The term "magnetic crystal" is defined as a particle
19 10A to 10,000 A in diameter comprised of iron oxide, iron
20 metal, cobalt metal, nickel metal, magnetic ferrites,
21 magnetic alloys, or mixed lattice magnetic metals or metal
22 oxides. The term "magnetic bead" is defined as a magnetic
23 crystal or population of crystals coated by an organic
24 moiety or polymer or inorganic moiety or polymer to form a
25 bead of 10A to 500,000 A in diameter. The term "magneto-
26 molecular switch" is defined as a cluster of magnetic
27 crystals or beads formed by the attachment of organic
28 moieties to the surface of the crystal or beads that link
29 the beads or crystals together via controllably degradable
30 chemical, complex, or ionic bonds.

31 As used herein the term:

32 "Polyalkylether" refers to polyethyleneglycol and
33 related homopolymers, such as polymethylethyleneglycol,
34 polyhydroxypropyleneglycol, polypropyleneglycol, poly-
35 methylpropyleneglycol, and polyhydroxypropyleneoxide, and
36 to heteropolymers of small alkoxy monomers, such as

1 polyethylene/polypropyleneglycol, such polymers having a
2 molecular weight of at least about 120 daltons, and up to
3 about 20,000 daltons.

4 "Amphipathic organic compound" refers to any organic
5 compound containing both a hydrophobic and hydrophilic
6 moiety.

7 "Amphipathic vesicle forming lipid" refers to any
8 lipid having a hydrophobic unit and hydrophilic unit, the
9 hydrophobic group typically including two acyl hydrocarbon
10 chains, the hydrophilic group containing a reactive
11 chemical group such as amine, acid, ester, aldehyde, or
12 alcohol group by which the lipid can be derivatized, e.g.
13 to a polyalkylether.

14 Summary of the Invention

15 This invention provides a method for preparing novel
16 precipitated inorganic oxide crystal particles of
17 substantially uniform particle size distribution. The
18 method comprises contacting aqueous solutions of an
19 inorganic salt and an inorganic base across a porous
20 membrane wherein the membrane contains a plurality of
21 pores which allows for precipitation of substantially
22 mono-dispersed inorganic oxide particles on one side of
23 the membrane and precipitation of a salt of the
24 corresponding base on a second side of the membrane.

25 When the inorganic oxide crystal particles produced
26 according to this method is an iron oxide particle of
27 reduced particle size (e.g. Fe_3O_4), which are non-
28 magnetic, they can be aggregated into one embodiment of
29 the magneto-molecular switch which comprises attachment of
30 organic moieties to the surface of the crystals that link
31 the crystal together to form controllably degradable
32 chemical, complex or ionic bonds. It has also been found
33 that aggregate clusters of crystals can be prepared by air
34 or inert gas drying of the crystal particles along with
35 several different solution encapsulation techniques.

36 In a further embodiment of the magneto-molecular

1 switch, the individual crystal particles or population of
2 crystals so produced are coated by polymer encapsulation,
3 adsorption of monomer followed by crosslinking, or by
4 applying organo-metallic polymer coatings which are
5 covalently bonded or adsorbed onto said particles, to form
6 a non-reversibly coated bead of 10A to 500,000 A in
7 diameter. Accordingly, the beads themselves can be
8 aggregated into controllably degradable bead clusters by
9 the organic moieties that may be present on the beads, or
10 by further attachment of organic moieties to the bead
11 surface, which in either case allow the beads to link
12 together to form controllably degradable chemical,
13 complex, or ionic bonds.

14 The present invention relates in one aspect to a
15 coated magnetically responsive particle comprising a
16 magnetic core particle comprising a magnetically
17 responsive metal, metal alloy or metal oxide and an
18 organo-metallic polymer coating covalently bonded to said
19 particle wherein the bonding does not depend on the
20 presence of hydroxy functionality on the surface of said
21 particle, and wherein the organo-metallic polymer coating
22 is capable of binding at least one type of bioaffinity
23 adsorbent. In addition to covalent bonding, the organo-
24 metallic polymer can be adsorbed. The coated magnetically
25 responsive particles have utility for either the
26 separation or directed movement of biological molecules
27 from a surrounding medium.

28 The organo-metallic polymer is formed from an organo-
29 metallic monomer, which is applied to the metal particle,
30 and thermally cross-linked in situ to form an adsorbed or
31 a covalently bound polymer coating. Organo-titanium
32 polymers are preferred, however, organo-metallic polymers
33 formed from coordinate complexes of other transition
34 metals, such as zirconium (Zr), hafnium (Hf), vanadium
35 (V), tantalum (Ta) and niobium (Nb) or post-transition
36 metals, such as tin (Sn) and antimony (Sb), can be used.

1 A wide variety of bioaffinity adsorbents can be covalently
2 bonded to the organo-metallic polymer coating through
3 selected coupling chemistries.

4 More particularly, the invention relates to methods
5 for the preparation of magnetically responsive particles
6 comprising a metal, metal alloy or metal oxide core and an
7 organo-metallic coating having an aliphatic moiety and an
8 organic functionality to which a variety of organic and/or
9 biological molecules can be coupled. The particles,
10 coupled or uncoupled, can be dispersed in aqueous media
11 forming a colloidal dispersion which is stable, that is,
12 the particles resist rapid gravitational settling. The
13 particles can be reclaimed from the media by applying a
14 magnetic field.

15 Preferably, the particles are superparamagnetic; that
16 is, they exhibit no reminent magnetization after removal
17 of a magnetic field which allows the particles to be
18 redispersed without magnetic aggregate formation.

19 The organo-metallic coated magnetically responsive
20 particles of the invention may be coupled through the
21 organic functionality to biological or organic molecules
22 with affinity for, or the ability to adsorb, or which
23 interact with, certain other biological or organic
24 molecules. Particles so coupled may be used in a variety
25 of in vitro or in vivo systems involving separations steps
26 or the directed movement of coupled molecules to
27 particular sites, including immunological assays, other
28 biological assays, biochemical or enzymatic reactions,
29 affinity chromatographic purification, cell sorting and
30 diagnostic and therapeutic uses.

31 In connection with the above, and in a further aspect
32 of the present invention, a method of measuring analytes
33 in a sample is disclosed comprising the steps of: (a)
34 contacting a sample containing an unknown concentration of
35 the analyte with a known amount of a labeled analyte in
36 the presence of magnetic particles comprising: (1) a

1 magnetic core particle comprising a magnetically
2 responsive metal, metal alloy or metal oxide; and (2) an
3 organo-metallic polymer coating covalently bonded to said
4 particle wherein the bonding does not depend on the
5 presence of hydroxy functionality on the surface of said
6 particles, and wherein said organo-metallic coating has a
7 bioaffinity adsorbent covalently coupled thereto, said
8 bioaffinity adsorbent is capable of binding to or
9 interacting with both the unlabeled and the labeled
10 analyte; (b) maintaining the mixture in step (a) under
11 conditions sufficient for said binding or interaction to
12 occur; (c) magnetically separating the magnetic particles;
13 and (d) measuring the amount of label associated with the
14 magnetic particles and determining the concentration of
15 analyte in solution.

16 The present organo-metallic coated magnetic particles
17 provide superior composition, size, surface area, coupling
18 versatility, settling properties, and magnetic behavior
19 for use in biological separations. The magnetic particles
20 of this invention are suitable for many of the assays,
21 enzyme immobilization, cell sorting and affinity
22 chromatography procedures reported in the literature and,
23 in fact overcome many of the problems associated with
24 particle settling and reuse experienced in the past with
25 such procedures.

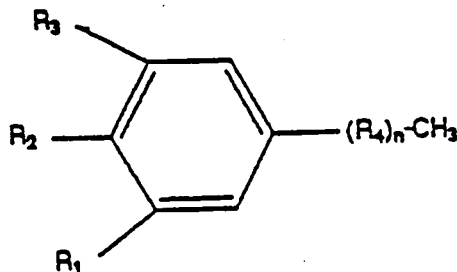
26 It has now been found that the inorganic oxides of
27 substantially uniform particle size can be used to prepare
28 a liposome composition comprising a substantially uniform
29 size inorganic core coated with an amphipathic organic
30 compound and further coated with a second amphipathic
31 vesicle forming lipid. The inorganic core is again
32 prepared by contacting aqueous solutions of an inorganic
33 salt and an inorganic base across a porous membrane
34 wherein the membrane contains a plurality of pores which
35 allows for precipitation of substantially monodispersed
36 size inorganic oxide particles on one side of the membrane

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1 and precipitation of a salt of the corresponding base on a
2 second side of the membrane. Inorganic cores are also
3 prepared by the reaction of metallocenes with aqueous
4 metal hydroxide slurries followed by milling to uniform
5 particle size. The class of inorganic cores include
6 Fe_3O_4 , Fe_2O_3 , Al_2O_3 , TiO_2 , ZnO , FeO , and Fe .

7 The amphipathic vesicle forming lipid is preferably a
8 lipid having two hydrocarbon chains, including acyl
9 chains, and a polar head group. Included in this class
10 are the phospholipids, such a phosphatidylcholine (PC),
11 phosphatidic acid (PA), phosphatidylinositol (PI),
12 sphingomyelin (SM), and the glycolipids, such as
13 cerebroside and gangliosides.

14 The amphipathic vesicle forming lipid can also be a
15 novel synthetic phenyl lipid compound having the
16 structural formula:



23 wherein two of R_1 , R_2 and R_3 represent a saturated or
24 unsaturated straight-chain or branched chain alkyl or acyl
25 group, the other being hydrogen, therein providing at
26 least two hydrocarbon chains attached to the phenyl
27 moiety, wherein the two hydrocarbon chains are typically
28 between about 14-22 carbon atoms in length, and have
29 varying degrees of unsaturation. R_4 represents the
30 repeating unit of either a poly(alkylene oxide) polymer,
31 preferably ethylene, propylene and mixtures thereof, or
32 the repeating unit of poly(vinyl alcohol). The number of
33 alkylene oxide or vinyl alcohol groups in the polymer,
34 designated as n , may vary from 0 to about 200 or more.

35 In a further aspect, the invention includes an
36 inorganic core liposome composition for administering

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1 drugs via the bloodstream, comprising a substantially
2 uniform size inorganic core coated with an amphipathic
3 organic compound and further coated with 1-20 mole percent
4 of an amphipathic vesicle-forming lipid derivatized with a
5 hydrophilic polymer, and containing the compound in
6 liposome-entrapped form.

7 It has now also been found that liposome compositions
8 can be prepared to comprise a wave absorbing magnetic core
9 coated with an amphipathic organic compound and further
10 coated with a second amphipathic vesicle forming lipid.
11 In a preferred embodiment, the wave absorbing magnetic
12 core particles comprise ferrite or mixed ferrite
13 materials, preferably of a uniform, controllable size and
14 narrow size distribution, wherein the primary component,
15 the oxide, is of the formula $M_2(+3)M(+2)O_4$, wherein $M(+3)$
16 is Al, Cr or Fe, and $M(+2)$ is Fe, Ni, Co, Zn, Zr, Sr, Ca,
17 Ba, Mg, Ga, Gd, Mn or Cd. In a further aspect, the oxides
18 can be advantageously mixed with LiO, MaO and KO, or with
19 Fe_2O_3 and Fe_3O_4 .

20 The preparation of substantially uniform size oxides,
21 1 to 50,000 nm in diameter, is achieved by conversion of
22 hydrous oxide gels, in a multi-step process, wherein
23 alkali is added to individual $M(+3)$ and $M(+2)$ aqueous
24 solutions, which separately precipitate the corresponding
25 metal hydroxide. The two precipitates are then coarsely
26 mixed to provide micron size amorphous gel particles, or
27 the gels can be finally mixed by ball milling, for
28 example, to a particle size of about 100 Å in diameter.
29 These particles are then heated to effect dehydration, in
30 the presence of oxygen or air, wherein the dehydration
31 temperature, time of dehydration, and concentration of
32 oxygen or air operate to control the particle size of the
33 oxide crystals therein produced.

34 In a further aspect, the invention includes a process
35 for the treatment of cancer cells by application of
36 external electromagnetic energy capable of the generation

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1 of heat in intracellular particles to induce selective
2 thermal death of cancer cells comprising intravenously
3 injecting into the patient a wave absorbing magnetic core
4 particle coated with an amphipathic organic compound and
5 further coated with a second amphipathic vesicle forming
6 lipid, absorbing said coated wave absorbing magnetic core
7 particle intracellularly into the cancer cells, subjecting
8 the patient to an alternating electromagnetic field to
9 inductively heat the magnetic core particle and thereby
10 the cancer cells, and continuing the inductive heating of
11 said magnetic core particle to attain an increase in
12 intracellular temperature to selectively kill the cancer
13 cells.

14 Brief Description of the Figures

15 Fig. 1 is a drawing of a precipitation chamber used in
16 accordance with the present invention.

17 Fig. 2 illustrates the general liposome composition
18 comprising a substantially uniform size inorganic core
19 coated with an amphipathic organic compound and further
20 coated with an amphipathic vesicle forming lipid.

21 Fig. 3 is a reaction scheme for preparing a phenyl
22 lipid derivatized with polyethyleneglycol.

23 Fig. 4 illustrates the general liposome composition
24 comprising a wave absorbing magnetic core particle coated
25 with an amphipathic organic compound and further coated
26 with an amphipathic vesicle forming lipid.

27 Detailed Description of The Invention

28 The magnetically responsive particles of this
29 invention overcome problems associated with the size,
30 surface area, gravitational settling rate and magnetic
31 character of previously developed magnetic particles.
32 Gravitational settling times in excess of about 24 hours
33 can be achieved with the present magnetic particles. The
34 gravitational settling time is defined to be the time for
35 the turbidity of a dispersion of particles to fall by
36 fifty percent in the absence of a magnetic field gradient.

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The present magnetic particles comprise a core of a magnetically responsive metal, metal alloy or metal oxide, coated with organo-metallic polymer, which is capable of binding reactive groups or agents, for example, chemically reactive groups, biologically reactive groups or bioaffinity agents. The organo-metallic polymer is adsorbed onto or covalently bound to the magnetic particle. The term "magnetically responsive particle" or "magnetic particle" is defined as any particle dispersible or suspendible in aqueous media without significant gravitational settling, and separable from suspension by application of a magnetic field.

The term "magnetic core" is defined as a crystal or group (or cluster) of crystals of a transition metal, alloy or magnetic metal oxide having ferrosipinel structure and comprising trivalent and divalent cations of the same or different transition metals or magnetic metal crystal group. Metals, alloys and oxides which are useful as magnetic core material in the present invention include the metals, alloys and oxides based on metals which appear in the Periodic Table in Groups 4a and b, 5a and b, 6a and 7a. These include, for example, divalent transition metals, such as iron, magnesium, manganese, cobalt, nickel, zinc and copper, alloys of these metals such as iron alloys or oxides (e.g., iron magnesium oxide, iron manganese oxide, iron cobalt oxide, iron nickel oxide, iron zinc oxide and iron copper oxide), cobalt ferrite, samarium cobalt, barium ferrite, and aluminum-nickel-cobalt and metal oxides including magnetite (Fe_3O_4), hematite (Fe_2O_3) and chromium dioxide (CrO_2). By way of illustration, a magnetic core may be

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comprised of a cluster of superparamagnetic crystals or iron oxide, or a cluster of superparamagnetic or ferromagnetic crystals of irons or oxide, or may consist of a single superparamagnetic or ferromagnetic crystal of
05 an iron oxide or metal alloy.

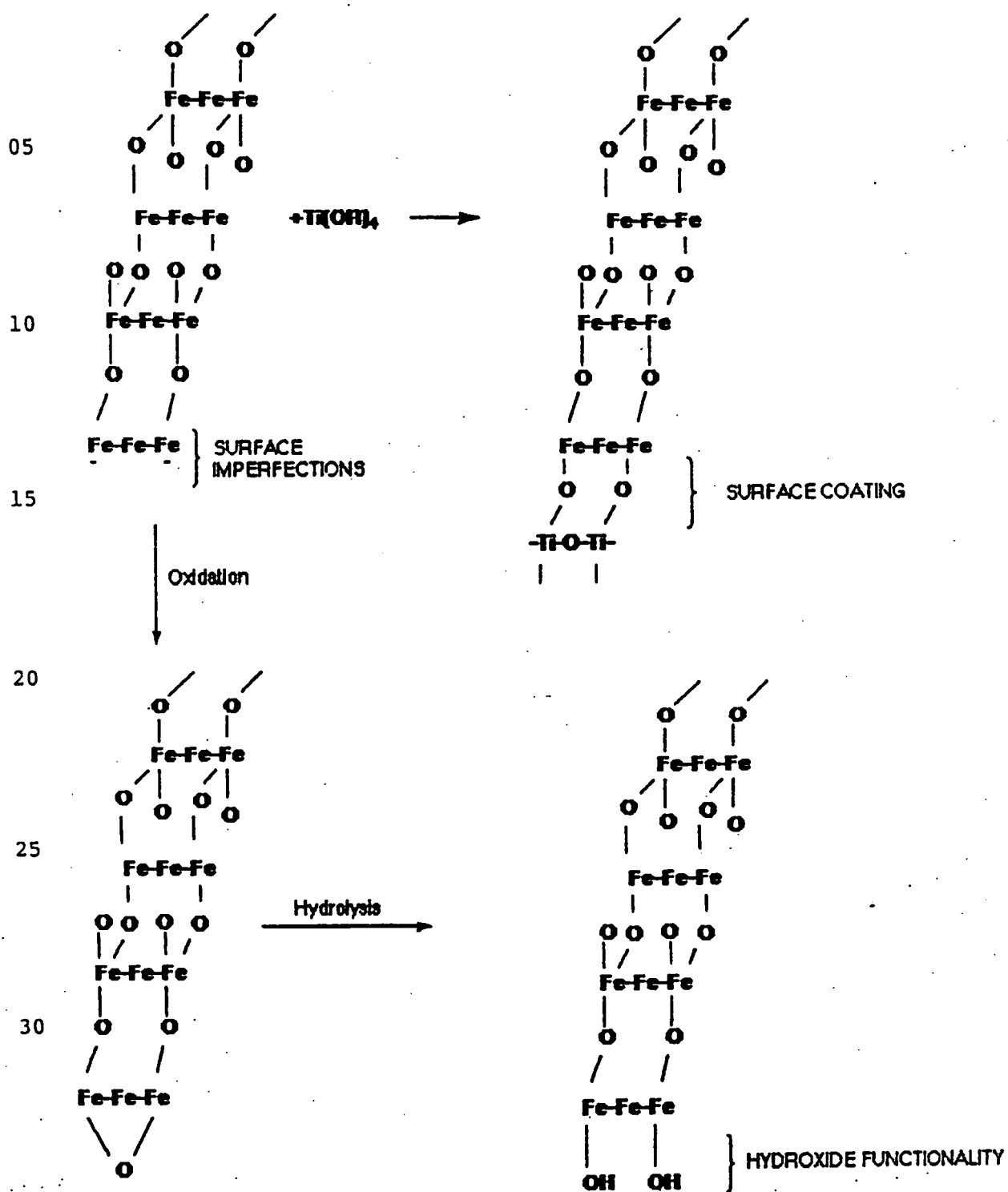
It has now been found that the Fe_3O_4 affords a crystal lattice which contains primarily trivalent iron (Fe^{+3}) at or near the surface of the crystal. These "surface trivalent" elements of the lattice contain imperfections
10 which make them available for direct covalent attachment of the organometallic compounds of the formula $\text{Ti}(\text{OR})_4$ according to the following general equation:

15

20

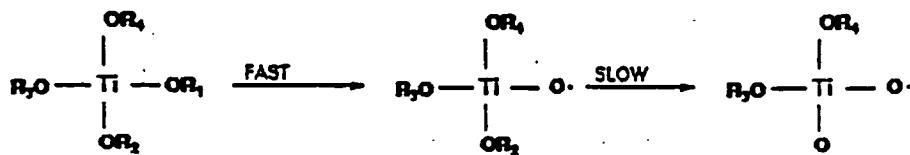
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It should be noted that the imperfections of the surface trivalent iron are somewhat short-lived, and if organo-metallic coating is delayed, oxidation and hydrolysis can occur causing the development of surface hydroxyls which preclude direct covalent attachment of the organo-metallic moiety. For example, freshly made Fe_3O_4 will spontaneously react; Fe_3O_4 material after 24 hours reacts but requires about 1 hour of dwell time; after 48 hours the coupling reaction takes place very slowly and is generally incomplete.

Organo-metallic compounds are preferably of the formula $\text{Ti}(\text{OR})_4$ wherein R is an alkyl group and the dissociation to the reactive component follows the following general reaction criterion:



Accordingly, R_1 , R_2 , R_3 and R_4 are selected so that rapid dissociation of the first radical (R_1) is fast, and dissociation of subsequent radicals (R_2 - R_4) is slow. It has been found that when the radicals R_1 - R_4 are collectively alkyl type, the dissociation is linear with respect to the length of the chain (the shorter the chain, the faster the dissociation). Therefore it is possible to shift the reactivity of such organo-metallic compounds by simply replacing shorter alkyl substituents with longer alkyl substitution. It has also been found that when R is an aryl moiety, dissociation is relatively slow. Other

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moieties (e.g. esters, ketones) have been found to provide intermediate dissociation constants.

The present particles are preferably between about 0.003 and about 1.5 microns in diameter, and have a surface area of from about 50 to 150 meters/gm, which provides a high capacity for coupling of a bioaffinity adsorbent, chemical or biochemical reactive group. Magnetic particles of this size range overcome the rapid settling problems of larger particles, but obviate the need for large magnets to generate the magnetic fields and magnetic field gradients required to separate smaller particles. For example, magnets used to effect separations of the magnetic particles of this invention need only generate magnetic fields between about 100 and about 1000 Oersteds. Such fields can be obtained with permanent magnets which are smaller than the container which holds the dispersion of magnetic particles and, thus, are suitable for benchtop use.

Particles with superparamagnetic behavior are preferred since superparamagnetic particles do not exhibit the magnetic aggregation associated with ferromagnetic particles and permit redispersion and reuse. The term "superparamagnetism" is defined as that magnetic behavior exhibited by iron, cobalt, nickel or other metal alloys or metal oxides having a crystal size of less than about 300A, which behavior is characterized by

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responsiveness to a magnetic field without remnant magnetization.

05 Ferromagnetic particles may be useful in certain applications of the invention. The term "ferromagnetism" is defined as that magnetic behavior exhibited by iron, iron alloys or iron oxides with a crystal size greater than about 500A, which behavior is characterized by responsiveness to a magnetic field with a remnant magnetization of greater than about 10 gauss upon removal
10 of the magnetic field.

The particles or crystals are then coated with an organo-metallic monomer material capable of adsorptive or covalently bonding to the magnetic particles. Organo-metallic monomers useful for the present coated particles
15 are organic coordinate complexes of selected transition and/or post transition metals which are capable of forming a stable coordination compound, and organic ligands, which can be adsorbed onto or covalently bound to the magnetic particle and, crosslinked in situ on the
20 particle surface, thereby forming the organo-metallic polymer coating. The organo-metallic monomer must be able to be functionalized or derivatized in a manner that allows the polymer formed therefrom to form covalent bonds with bioaffinity or chemical affinity adsorbents.
25 For this purpose, the organo-metallic polymer is post-functionalized or derivitized with an aliphatic "spacer arm" which is terminated with an organic functional group capable of coupling with bioaffinity adsorbents. The "spacer arm" is an aliphatic hydrocarbon having from
30 about 2 to about 60 atoms, e.g., carbon, nitrogen and/or oxygen atoms. The purpose of the spacer arm is to

provide a non-reactive linker (or spacer) between the organic group which reacts with the chemical group, biochemical group or bioaffinity adsorbent and the polymer chain, and to impart an appropriate degree of hydrophilic/hydrophobic balance to the surface of the coated particle. The organic group is generally a reactive group such as an amine (NH_2), carboxyl group (COOH), cyanate (CN), phosphate (PO_3H), sulfate (SO_3H), thiol (SH), hydroxyl (OH) group, vinyl ($\text{C}=\text{C}$), nitrate (NO_2), aldehyde, epoxide, succinamide or anhydride group coupled to an aliphatic or aromatic moiety.

Particularly useful organo-metallic compounds are coordinate complexes formed from selected transition metals (e.g., Ti, Zr, Hf, V, Zn, Cd, Mn, Te, Re, Ta, Nb) and/or post-transition metals (e.g., Sn, Sb, Al, Ga, In, Ge). Organo-titanium compounds are particularly preferred. Organo-titanium compounds which are useful including, for example, titanium-tetra-isopropoxide, amino-hexyl-titanium-tri-isopropoxide, amino-propyl-titanium-tri-isopropoxide and carboxyl-hexyl-titanium-tri-isopropoxide. In one embodiment of the present invention, amino-hexyl-titanium-tri-isopropoxide is coated onto the magnetic particle of choice, and thermally crosslinked to form an organo-titanium polymer coating having an aliphatic spacer arm (the hexyl moiety) and organic functional group (the amine group).

The coated particle is post-functionalized, if necessary, in a manner that allows the organo-metallic polymer to form covalent bonds with bioaffinity or chemical affinity adsorbents. In one embodiment of the present method, an organo-titanium polymer, such as

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titanium-tetra-isopropoxide which lacks the spacer arm and organic functional group, is coated onto the magnetic particle of choice and partly crosslinked at about 40°C for a period of time sufficient to allow the

05 organotitanium polymer to become adsorbed onto the particle surface. The organotitanium coated magnetic particle is then activated by reaction with an agent such as 1-hydroxy-6-amino hexane, to form the amino-hexyl-titanium-tri-isopropoxide. The coating is then

10 crosslinked at elevated temperatures to form an organotitanium polymer coating having an aliphatic spacer arm and an organic functionality (i.e., the amine group). The functionalized particle can then be reacted or coupled, with the bioaffinity adsorbent of choice.

15 The magnetic core particles are prepared according to the following general procedure: metal salts are precipitated in a base to form fine magnetic metal oxide crystals. The crystals are redispersed, then washed in water and in an electrolyte. Magnetic separation can be

20 used to collect the crystals between washes if the crystals are superparamagnetic.

In one embodiment of the present invention, superparamagnetic iron oxide particles are made by precipitation of divalent (Fe^{2+}) and trivalent (Fe^{3+}) iron salts, for example, ferrous ammonium sulfate, $\text{Fe}_2(\text{NH}_4)(\text{SO}_4)$ and ferric sulfate, $\text{Fe}_2(\text{SO}_4)_3$, in aqueous base. The ratio of Fe^{2+} and Fe^{3+} and counterion can be varied without

25 substantial changes in the final product by increasing the amount of Fe^{2+} while maintaining a constant molar amount of iron. Counterions including nitrate, sulfate, chloride or hydroxide are useful in the method. A

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Fe²⁺/Fe³⁺ ratio of about 2:1 to about 4:1 is useful in the present invention; a ratio of about 2:1 Fe²⁺:Fe³⁺ is particularly useful. An Fe²⁺/Fe³⁺ ratio of 1:1 produces magnetic particles of slightly inferior quality to those
05 resulting from the higher Fe²⁺/Fe³⁺ ratios, the particle size is more heterogeneous than that resulting from Fe³⁺/Fe²⁺ of 2:1 or 4:1.

In this embodiment, aqueous solutions of the iron salts are mixed in a base, such as ammonium, sodium or
10 potassium hydroxide, which results in the formation of a crystalline precipitate of superparamagnetic iron oxide. The precipitate is washed repeatedly with water by magnetically separating and redispersing it until a neutral pH is reached. The precipitate is then washed
15 with about five equal portions of a water miscible solvent, such as acetone, methanol or ethanol that has been dried over molecular sieves to remove all of the water.

The repeated use of magnetic fields to separate the iron oxide from suspension during the washing steps is
20 facilitated by the superparamagnetic properties of the crystals. Regardless of how many times the particles are subjected to magnetic fields, they never become magnetically agglomerated and consequently, can be
25 redispersed by mild agitation. Ferromagnetic particles cannot be prepared by this washing procedure as they tend to magnetically aggregate after exposure to magnetic fields and cannot be homogeneously redispersed.

Other divalent transition metal salts such as
30 magnesium, manganese, cobalt, nickel, zinc and copper salts may be substituted for iron salts in the

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precipitation or milling procedure to yield magnetic metals or metal oxides. For example, the substitution of divalent cobalt chloride (CoCl_2) for FeCl_2 in the above procedure produced ferromagnetic metal oxide particles.

05 Ferromagnetic metal oxide particles such as those produced with CoCl_2 can be washed in the absence of magnetic fields by employing conventional techniques of centrifugation or filtration between washings to avoid magnetizing the particles. As long as the resulting

10 ferromagnetic metal oxides are of sufficiently small diameter to remain dispersed in aqueous media, they can also be coated with the organo-metallic polymer and coupled to bioaffinity adsorbents for use in systems requiring a single magnetic separation, e.g., certain

15 radioimmunoassays. Ferromagnetism limits particle usefulness in those applications requiring redispersion or reuse.

In another embodiment of the present invention, the magnetic core particles can be made by precipitating

20 metal powders and reducing the particle size by milling the resulting precipitate, for example, in a ball mill. In this process, the metal powder is precipitated from an aqueous solution of, for example, Fe^{+2} or Fe^{+3} salt with sodium borohydride. For example, an aqueous solution of

25 ferrous chloride (FeCl_2) is mixed with sodium borohydride (NaBH_4) to form a fine iron precipitate. The resulting properties of the metal powder are unaffected by the valance of the counter ion or iron metal salt selected. Complete precipitation occurs spontaneously upon

30 borohydride addition. The magnetic metal powder is then collected by filtration and washed with about five equal

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volumes of water to remove all soluble salts, then washed with five equal volumes of dried acetone to remove all residual water. The particle is added as an aqueous slurry in a concentration of about 1-25% to a commercial
05 ball mill filled half way with 1/4" stainless steel balls and milled for 3-30 days. At the completion of the milling period, a superparamagnetic metal slurry is formed and coated and functionalized as the superparamagnetic particles described in the previous section.

10 In another embodiment of the present invention, the magnetic core particles are made by reacting a metallocene, e.g., particulate ferrocene (dicyclopentadienyliron, $C_{10}H_{10}Fe$) with iron (II) hydroxide. In this embodiment, an aqueous ferrocene (or
15 other metallocene) slurry is prepared, and an aqueous slurry of iron (II) hydroxide is prepared separately. The ferrocene slurry is prepared, for example, by milling a mixture of ferrocene and water in a ball mill. The iron (II) hydroxide slurry can be prepared, for example, by
20 precipitating an aqueous solution of ferrous sulfate with ammonium hydroxide to form ferrous hydroxide. The two slurries are then combined and milled, for example, forming fine magnetite particles. Other metallocene compounds (e.g. nickelocene, cobaltocene) can be mixed
25 with the ferrocene to produce various magnetic ferrite particles. This process is described in detail in U.S. Patent No. 5,071,076, the teachings of which are hereby incorporated by reference.

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In one embodiment of the present invention, the coating around the magnetic core particle is amino-propyl-titanium-tri-isopropoxide. The polymerization is performed by redispersing the magnetic particle in an acetone solution, adding the organo-titanium monomer, then crosslinking with heat. The terms "coupled magnetically responsive particle" or "coupled magnetic particle" refer to any magnetic particle to which one or more types of bioaffinity adsorbents are coupled by covalent bonds, which covalent bonds may be amide, ester, ether sulfonamide, disulfide, azo or other suitable organic linkages depending on the functionalities available for bonding on both the coating of the magnetic particle and the bioaffinity adsorbents.

Preferred magnetically responsive particles of the present invention have metal oxide cores composed of clusters of superparamagnetic crystals affording efficient separation of the particles in low magnetic fields (100-1000 Oersteds) while maintaining superparamagnetic properties. Aggregation of particles is controlled during particle synthesis to produce particles which are preferably small enough to avoid substantial gravitational settling over times sufficient to permit dispersions of the particles to be used in an intended biological assay or other application. The advantage of having superparamagnetic cores in magnetically responsive particles is that such particles can be repeatedly exposed to magnetic fields. Superparamagnetic particles do not exhibit reminent magnetization and have no coercive strength, and, therefore, do not magnetically aggregate, thus, the particles can be redispersed and

reused. Even after coating, preferred particles of the invention having cores made up of clusters of crystals exhibit a remarkably high surface area per unit weight and a generally corresponding high coupling capacity, which indicates that such particles have an open or porous structure.

The bioaffinity adsorbents can be covalently bonded to the organo-metallic coated magnetic particles of this invention by conventional coupling chemistries. Several coupling reactions can be performed. For example:

(a) If the ligand to be coupled contains an amino group, it can be coupled directly to the activated organo-metallic polymer. If a different functionality is desired, it can be introduced, for example, by adding a spacer arm containing the functionality by sequential reaction of the organo-metallic polymer (e.g., titanium-tetra-isopropoxide) with any omega-functional higher molecular weight alcohol. The amino group on the ligand can then be coupled to the free functional group on the spacer arm; or

(b) If the ligand contains an aldehyde group instead of an amino group, it can be coupled directly to the free amino group of an amino alkane (that is, an alkane spacer arm having an amino functionality) on the coated magnetic particle.

The term "bioaffinity adsorbent" is defined as any biological or other organic molecule capable of specific or nonspecific binding or interaction with another biological molecule, which binding or interaction may be referred to as "ligand/ligate" binding or interaction and is exemplified by, but not limited to, antibody/antigen,

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antibody/hapten, enzyme/substrate, carrier protein/substrate, lectin/carbohydrate, receptor/hormone, receptor/effector or repressor/inducer bindings or interactions.

The coupled organo-metallic coated magnetic
05 particles of the present invention can be used in immunoassays or other binding assays for the measurement of analytes in solution. The term "immunoassay" is defined as any method for measuring the concentration or amount of an analyte in a solution based on the immunological
10 binding or interaction of a polyclonal or monoclonal antibody and an antigen, which method (a) requires a separation of bound from unbound analyte; (b) employs a radioisotopic, fluorometric, enzymatic, chemiluminescent or other label as the means for measuring the bound
15 and/or unbound analyte; and (c) may be described as "competitive" if the amount of bound measurable label is generally inversely proportional to the amount of analyte originally in solution or "non-competitive" if the amount of bound measurable label is generally directly proportional to the amount of analyte originally in the solution. Label may be in the antigen, the antibody, or in
20 double antibody methods, the second antibody. Immunoassays are exemplified by, but are not limited to, radioimmunoassays (RIA), immunoradiometric assays (IRMA),
25 fluoroimmunoassays (FIA), enzyme immunoassays (EIA), and sandwich method immunoassays. The analyte or the bioaffinity adsorbent can include, for example, antibodies, antigens, haptens, enzymes, apoenzymes, enzymatic substrates, enzymatic inhibitors, cofactors, nucleic
30 acids, binding proteins, carrier proteins, compounds bound by binding proteins, compounds bound by carrier

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proteins, lectins, monosaccharides, polysaccharides, hormones, receptors, repressors and inducers.

Such assays are preferably carried out by mixing a sample containing an unknown concentration of analyte with a known amount of labeled analyte in the presence of magnetic particles coupled to a bioaffinity adsorbent capable of binding to, or interacting with, both unlabeled and labeled analyte, allowing the binding or interaction to occur, magnetically separating the particles, measuring the amount of label associated with the magnetic particles and comparing the amount of label to a standard curve to determine the concentration of analyte in the sample.

The term "binding assay" or "non-immune assay" is defined as any method for measuring the concentration or amount of an analyte in solution based on the specific or nonspecific binding or interaction, other than antibody/antigen binding or interaction, or a bioaffinity adsorbent and another biological or organic molecule, which method (a) requires a separation of bound from unbound analyte; (b) employs a radioisotopic, fluorometric, enzymatic, chemiluminescent or other label as the means for measuring the bound and/or unbound analyte; and (c) may be described as "competitive" if the amount of bound measurable label is generally inversely proportional to the amount of analyte originally in solution or "non-competitive" if the amount of bound measurable label is generally originally in solution.

The magnetic organo-metallic-coated particles of this invention are useful in immobilized enzyme systems, particularly where enzyme recycling is desired. The term

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"immobilized enzyme system" is defined as any enzymatically catalyzed biochemical conversion or synthesis or degradation wherein the enzyme molecule or active site thereof is not freely soluble but is adsorptively or covalently bound to a solid phase support, which support is suspended in or contacted with the surrounding medium and which may be reclaimed or separated from said method. In this embodiment, enzymatic reactions are carried out by dispersing enzyme-coupled magnetic particles in a reaction mixture containing one or more substrates, under conditions sufficient for the reaction between the enzyme and substrate to occur, magnetically separating the enzyme-magnetic particle from the reaction mixture containing products and unreacted substrates and, if desired, redispersing the particles in fresh substrates thereby reusing the enzyme.

Affinity chromatography separations and cell sorting can be performed using the magnetic particles of this invention. The term "affinity chromatography" is defined as a method for separating, isolating, and/or purifying a selected molecule from its surrounding medium on the basis of its binding or interaction with a bioaffinity adsorbent adsorptively or covalently bound to a solid phase support, which support is suspended in or contacted with the surrounding medium and which may be reclaimed or separated from said medium by dispersing bioaffinity adsorbent coupled magnetic particles in solutions or suspensions containing molecules or cells to be isolated and/or purified, allowing the bioaffinity adsorbent and the desired molecules or cells to interact, magnetically separating the particles from the solutions or suspension

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and recovering the isolated molecules or cells from the magnetic particles.

It is further contemplated that the organo-metallic coated magnetic particles of this invention can be used
05 in in vivo systems for the diagnostic localization of cells or tissues recognized by the particular bioaffinity adsorbent coupled to the particle and also for magnetically directed delivery of therapeutic agents coupled to the particles to pathological sites.

10 Magnetic separation times of less than about ten minutes can be achieved with magnetic particles of the invention by contacting a vessel containing a dispersion of the particles with a pole face of a permanent magnet no larger in volume than the volume of the vessel.

15 Magnetic separation time is defined to be the time for the turbidity of the dispersion to fall by 95 percent.

Furthermore, the use of functionalized organo-metallic polymers as the coating surrounding the metal oxide core of the magnetic particles described herein
20 make possible the coupling of a wide variety of molecules under an equally wide variety of coupling conditions compared to other magnetic particle coatings known in the art with more limited coupling functionalities.

The invention is further illustrated by the following
25 Examples.

EXAMPLES

Example 1: Preparation of Superparamagnetic Magnetite Particles

200 grams (1.58 moles) of ferrous chloride (VWR Scientific) and 325 grams (2.0 moles) of ferric chloride

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were dissolved in 3 liters of water. 2000 grams of ammonium hydroxide (VWR Scientific) concentrate were added at a rate of 50 ml/minute under constant agitation, during which time the temperature of the solution was kept between 25 and 40°C. After the addition of the ammonium hydroxide was complete, the magnetic particle (Fe_3O_4) aqueous slurry was allowed to cool to room temperature.

10 Example 2: Preparation of Amino-Hexyl-Titanium-Tri-Isopropoxide

0.1 moles of titanium-tri-isopropoxide (Tyzor TPT Dupont, Wilmington, DE) and 0.1 moles of 6-amino-1-hexanol were added to a 50 ml beaker and stirred at room temperature for 1 minute to form 0.1 mole of amino-hexyl-titanium-tri-isopropoxide. The reaction mixture was heated to 70°C for 10 minutes to evaporate the isopropyl alcohol formed during the reaction.

The material was cooled to room temperature and used as a monomer in making the tetravalent titanium organo-metallic coating in Example #3.

Example 3: Preparation of Amine Functional Organo-titanate Coated Magnetic Particle

According to the procedure set out in Example 1, 4 moles FeCl_3 and 2 moles of FeCl_2 were dissolved in 4 L of distilled water and precipitated with 16 moles of ammonium hydroxide. The precipitate was washed 5 times with water and 3 times with acetone. N,N-dimethylformamide (DMF) was added to the precipitate in the following ratio: 10 ml of DMF per gram of Fe_3O_4 . The mixture was loaded into a Eiger Mill and milled

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continuously for 10 minutes. The mixture was then transferred to a beaker and heated with stirring for 30 minutes at 100°C. The amine functional organo-titanate prepared in Example 2 was immediately added after
05 preparation with constant stirring to the mixture in a ratio of 1 g dry Fe_3O_4 per 3 g of amine functional organo-titanate.

This mixture was then heated with stirring for 20 minutes at 65°C and then passed through the Eiger Mill
10 for two passes. The resulting material was washed five times with water, the coated particles were collected with an external magnetic field of 2000 gauss and the aqueous waste was decanted.

15 Example 4: Preparation of An Alternating Functional-Non Functional Organo-Titanate Monomer

The procedure described in Example 2 was followed except that the organo-titanate was reacted with a comixture of amino-functional hexanol and hexanol to produce a monomer having reduced amine functionality.
20 Hexanol and 6-amino-1-hexanol in a molar ratio of 6:1 were mixed in a 50 ml beaker for one minute. Tyzor TPT was added to the alcohol mixture in the ratio of 1 mole of alcohol per mole of Tyz or TPT. The reaction mixture was stirred for one minute, heated to 70°C for 10 minutes
25 to evaporate the isopropyl alcohol produced by the reaction and cooled to room temperature. The resulting compound was an organotitanate, 6-amino-hexyl-titanium-tri-isopropoxide having alternating non-functional hexyl groups, that is, hexyl chains lacking the amino group.
30 The weight ratios of 6-amino-1-hexanol:Tyzor TPT:hexanol

were 1:26:9.6. This compound was used as a monomer to make an organo-titanium coating as described in Example 5.

05 Example 5: Preparation of Amine Functional Organo-
titanate Magnetic Particles

The procedure described in Example 3 was followed except that the amine-functional organo-titanate was the material prepared in Example 5. The mixture of magnetic particles and organo-titanate monomer was heated to 95°C for one hour with constant stirring and milled in an Eiger Mill for 4 minutes. The mixture was washed nine times with water. Adipic acid was added in the ratio of 0.5 moles of adipic acid per mole of total particles. One mole of carbodiimide (CDI) was added, and the mixture was mixed for 30 minutes on a ball mill. 1,6 hexane-diamine was added in the ratio of 0.5 moles of 1,6 hexane-diamine per mole of total particles. One mole of CDI was added and the mixture was mixed for 30 minutes. The resulting material was washed five times with water, the particles were collected using an external magnetic field of 2000 gauss and the aqueous waste was decanted.

Example 6: Preparation of Subdomain Magnetite Particles
by Reaction of Particulate Ferrocene and
Iron(II) Hydroxide

25 A 100 g of a slurry containing 20% ferrocene (by weight) (dicyclopentadienyliron; Strem Chemical Co., Newburyport, MA) in water was prepared by mixing the ferrocene with the water. The slurry was added to a commercial ball mill. The mill was filled halfway with 30 1/2" stainless steel balls and the slurry was milled for a

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period of 2 hours.

A second ferrous hydroxide slurry (iron (II) hydroxide) was made according to the following procedure. An aqueous solution containing 20g of ferrous sulfate
05 (VWR Scientific) was precipitated using 50g of ammonium hydroxide concentrate to form gelatinous ferrous hydroxide. The gel was filtered and the filtrate washed with 5 to 100g volumes of water. The washed gel was then made into a 10% aqueous slurry and milled as previously
10 described for 5 hours.

The ferrocene and hydroxide slurries were mixed, and the mixture was milled for one day to form fine Fe_3O_4 particles. The particles were about 100 Å in diameter and were responsive to a magnetic field. These particles
15 can be coated as described in Examples 2-5 above.

Example 7: Preparation of Subdomain Nickel-Ferrite Particles

Subdomain nickel-ferrite particles were prepared according to the procedure set out in Example 6, except
20 that a mixture of 50g a 20% nickelocene slurry (dicyclopentadienylnickel; Strem Chemical Co., Newburyport, MA) and 50g of a 20% ferrocene slurry were used in lieu of the 100g of the ferrocene slurry in Example 6. Magnetically responsive nickel-ferrite
25 particles having a particle size of about 100 Å were produced by this method.

Example 8: Preparation Subdomain Cobalt-Ferrite Particles

Subdomain cobalt-ferrite particles were prepared
30 according to the procedure set out in Example 6, except that a mixture of 50g of a 20% (by wt.) cobaltocene

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slurry (dicyclopentadenylcobalt; Strem Chemical Co., Newburyport, MA) and 50g of the ferrocene slurry were used in lieu of 100g of the ferrocene slurry in Example 6. Magnetically responsive cobalt-ferrite particles
05 having a particle size of about 100 Å were produced by this method.

Example 9: Preparation of Subdomain Metal Particles by Sodium Borohydride Reduction and Size Reduction by Milling

10 200 gm (1.58 moles) of ferrous chloride was dissolved in 1 liter of water. 500 gm of dry sodium borohydride were added to the solution to form a fine iron powder precipitate. The precipitate was washed with water and collected by filtration. The filtered powder
15 was resuspended in water and re-filtered. The washing procedure was done 4 additional times. On the final suspension, the slurry was adjusted to a concentrate of 20% and milled as described in Example 6 for a period of 75 days to produce particles with a mean diameter of less
20 than 50 Å.

1 Description of the Sub 100A Ferrite Particle

2 Sub 100A ferrites have been prepared by the co-
3 precipitation of metal(+2) and metal(+3) salts in aqueous
4 solutions with aqueous base across a porous or dialysis
5 membrane. The metal salt solutions are put into a
6 dialysis bag and the bag is sealed. The bag containing
7 the metal salt solution is then immersed in an aqueous
8 solution of base (i.e. ammonium hydroxide) over a period
9 of several minutes to several days, depending on the
10 concentration of the various reactants, and a precipitate
11 of metal oxide forms inside of the dialysis bag. The size
12 of the particles thus prepared is controlled by:
13 concentration of the metal salt solution; concentration of
14 the base solution; pore size of the membrane; temperature
15 of the various solutions; ionic strengths (or ionization
16 constant) of solutions; and the contact times of each
17 solution across the dialysis membrane.

18 It has further been discovered that metal oxide
19 particles of various controlled size can also be formed by
20 contacting an aqueous solution of metal salts with a
21 dialysis bag filled with aqueous base. In this case, the
22 desired metal oxide product will form outside of the
23 dialysis bag.

24 In a preferred embodiment, the inorganic base and the
25 inorganic salt solutions are maintained in large volume
26 chambers separated by a porous membrane. Accordingly,
27 large amounts of inorganic oxide of controlled particle
28 size can be produced. As can be seen from Figure 1, a
29 large volume chamber (10) contains a partition (12), a
30 semi-permeable membrane (14), an opening (16), a support
31 (18) for mounting of the membrane, and portals (20) for
32 draining. The metal salt solution is placed on the
33 membrane side of the chamber, such that the metal oxide
34 particles precipitate on that side of the large volume
35 chamber.

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1 It has also been discovered that the size of the
2 cationic moiety on the base side of the membrane controls
3 the size of the precipitated inorganic oxide particle so
4 produced near the surface of the membrane within the
5 inorganic salt solution. Apparently, the speed of
6 dissociation of the inorganic base is believed to be
7 controlled by the size of the cationic moiety; the larger
8 the cationic moiety the slower the dissociation to
9 cationic and anionic component. When the dissociation is
10 relatively slow, a relatively low concentration of anionic
11 moiety is present, providing a relatively low
12 concentration of anion diffusing across the porous
13 membrane and into the inorganic salt solution.
14 Accordingly, the cationic component (of the inorganic
15 salt) exists in large excess, thereby surrounding the
16 slowly diffusing anion, resulting in precipitation of many
17 small-sized inorganic oxide particles.

18 By contrast, if the cationic moiety of the inorganic
19 base is relatively small, the speed of dissociation is
20 relatively fast, providing a relatively large
21 concentration of anionic moiety diffusing across the
22 porous membrane and into the inorganic salt solution. At
23 the surface of the membrane within the inorganic salt
24 solution the cationic component (of the inorganic salt)
25 once again exists in large excess. Accordingly, while the
26 cationic component surrounds those anionic moieties which
27 have diffused across the membrane, the elevated
28 concentration of diffusing anionic moiety rapidly finds
29 its way to the cationic surface of such a growing
30 particle, so that a further layer of ionic bonding can
31 result, thereby producing larger overall particle size
32 prior to precipitation from solution.

33 It has been found, for example, that KOH in contact
34 with an aqueous solution of $\text{FeCl}_2/\text{FeCl}_3$ affords iron oxide
35 particles (Fe_3O_4) that are smaller in size as compared to
36 iron oxide particles produced when LiOH is employed as the

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1 inorganic base. This would comport with the above insofar
2 as the K⁺ ion is known to be relatively larger than the
3 Li⁺ ion.

4 With respect to the foregoing, NH₄OH, KOH, LiOH, NaOH
5 and other hydroxides formed by elements in group Ia of the
6 periodic table serve as suitable inorganic base compounds.
7 Inorganic salt solutions based on mixtures of the type
8 M⁽⁺³⁾Y/M⁽⁺²⁾Y include those wherein Y is selected from the
9 group consisting of Cl, Br, I, SO₄, NO₃ and PO₄. M can be
10 selected from the group consisting of Fe, Co, Ni, Zn, Mn,
11 Mg, Ca, Ba, Sr, Cd, Hg, Al, B, Sc, Ga, V and In. The
12 preferred inorganic salts are those which are readily
13 productive in an aqueous medium of an anion and a cation
14 which can combine with the aforementioned diffusing
15 hydroxide anion to form an inorganic oxide.

16 Accordingly, inorganic oxide particles of the formula
17 M₃O₄ are prepared wherein M is selected from the group
18 consisting of Fe, Co, Ni, Zn, Mn, Hg, Ca, Ba, Sr, Cd, Hg,
19 Al, B, Sc, Ga, V and In and mixtures thereof. It will
20 also be appreciated that for a given M₃O₄ particle, the
21 metal (M) may often be a combination of different
22 oxidation states of the same metal component. For
23 example, and in the preferred embodiments, Fe₃O₄ particles
24 are prepared and represent a mixed Fe⁽⁺²⁾Fe⁽⁺³⁾ oxide of
25 the formula [Fe⁽⁺²⁾][Fe⁽⁺³⁾]₂O₄.

26 With respect to the foregoing, reference is made to
27 the following:

28 I. The Effect of Alternative Base Counter Ions

29 The effect of alternative base counter ions on crystal
30 properties such as size, distribution, magnetics, etc. was
31 established as follows: Three experiments were conducted.
32 All experimental conditions were identical except for the
33 type of base. Experiment A utilizes NaOH, B with LiOH and
34 C with KOH. For each experiment: 1. Wash a Spectra/Por®
35 5 dialysis membrane (cellulose ester based membrane
36 available from Spectrum Medical Industries, Inc.) and

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1 secure over the opening in the dialysis chamber; 2. Fill
 2 both sides of the tank with 20 liters of distilled H₂O (at
 3 room temperature); 3. Dissolve 12.5g FeCl₂·4H₂O in 2
 4 liters of distilled H₂O. Add 20 g FeCl₃ and stir until
 5 dissolved; 4. Decant all iron solution into the membrane
 6 side of chamber; 5. For A dissolve 55g NaOH in 2 liters
 7 of distilled H₂O. For B dissolve 55g LiOH in 2 liters of
 8 distilled H₂O. For C dissolve 60.6g KOH in 2 liters of
 9 distilled H₂O. Decant base solution into opposite side of
 10 dialysis chamber. After 70 hours contact time, remove the
 11 crystal precipitate solution for evaluation. The results
 12 are listed below in Table 1.

Table 1

Sample	Base Ion	Crystal Size(A)	Cluster Size(A)	Magnetics (Gauss)	Iron Conc. (mg/ml)	% Total Solids
A	Na	60-80	300	340	7.0	1
B	Li	120-140	170-250	275	6.44	1
C	K	40	500	187	6.10	1

II. The Effect of Base Concentration

20 The effect of base concentration on crystal properties
 21 such as size, distribution, magnetic response, etc. was
 22 established as follows: Two experiments were conducted.
 23 All experimental conditions were identical except for base
 24 concentration. Experiment A was conducted at 0.5% NaOH.
 25 Experiment B was conducted at 0.25% NaOH. For each
 26 experiment: 1. Wash a Spectra/Por[®] 5 dialysis membrane and
 27 secure over the opening in the dialysis chamber; 2. Fill
 28 both sides of the tank with 20 liters of H₂O (at room
 29 temperature); 3. Dissolve 12.5g FeCl₂·4H₂O in 2 liters of
 30 distilled H₂O. Add 20g FeCl₃ and stir until dissolved; 4.
 31 Decant all iron solution into the membrane side of chamber;
 32 5. For concentration A: Dissolve 120g NaOH in 2 liters
 33 distilled H₂O. Decant into opposite side of tank; For
 34 concentration B: Dissolve 55g NaOH in 2 liters distilled
 35 H₂O. Decant into opposite side of tank; 6. After 70-80
 36 hours contact time remove iron solution and precipitated
 37 crystals for evaluation. The results are listed below in

1 Table 2.

2 Table 2

3

4	5	6	7	8	9	10
Sample	Crystal Size(A)	Cluster Size(A)	Magnetics Gauss	Iron Conc.	% Total Solids	
6 A	70-80	500	360	7.0mg/ml	1.0	
7 B	60-80	300	340	0.39mg/ml	0.085	

8 It has also been found that the size of the particles
9 may be effected by the following additional variable: the
10 temperature of the solutions; whether the particles formed
11 are removed (including magnetic removal, if the particles
12 are of the appropriate size) from the immediate surface of
13 the membrane; the pore size of the membrane; and whether
14 or not the solutions are stirred. With respect to the
15 pore size, membranes of different molecular-weight cut-
16 offs (MWCO) have been examined. The MWCO represents a
17 limit on the size of the molecule allowed to pass through
18 the pore. MWCO's between 1000 and 500,000 have been
19 investigated. The smaller the MWCO, the smaller the
20 inorganic oxide produced.

21 Description of Magnetic Clusters

22 Iron oxide, for example, has been prepared using this
23 technique in sizes of 80A, 50A and 20A, all with a narrow
24 (+/-10%) particle size distribution. A product that
25 agrees with x-ray diffraction patterns for Fe₃O₄ has been
26 prepared in 100, 80, 50 and 20A crystal sizes. The supra
27 50A particles of Fe₃O₄ have domain magnetization, when
28 measured by a Vibrating Sample Magnetometer (VSM), of 5660
29 gauss. This result is in agreement with the literature.
30 The sub 50A Fe₃O₄ crystals surprisingly have a very low
31 magnetization. In fact, crystals of 20A Fe₃O₄ have domain
32 magnetization of less than 100 gauss. This low
33 magnetization observed in sub 50A Fe₃O₄ crystals is likely
34 the result of having insufficient mass for spin coupling
35 and the absence of domain wall formation.

36 Surprisingly, when non-magnetic sub 50A crystals of
37 Fe₃O₄ are clustered together to form aggregates of 250A or

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1 greater, the aggregate particles are strongly magnetic.
2 Aggregate particles of 500A or greater in diameter, when
3 measured by VSM, have domain magnetizations in excess of
4 4000 gauss.

5 It has been further discovered that if the aggregates
6 of magnetic crystals are returned to non-aggregated unit
7 sub 50A crystal size, the effect is reversed, that is, the
8 magnetization is returned to nominally 0.

9 The exact size at which the onset of superparamagnetic
10 behavior occurs in the unit crystal, is a function of the
11 crystal structure, shape, and composition.

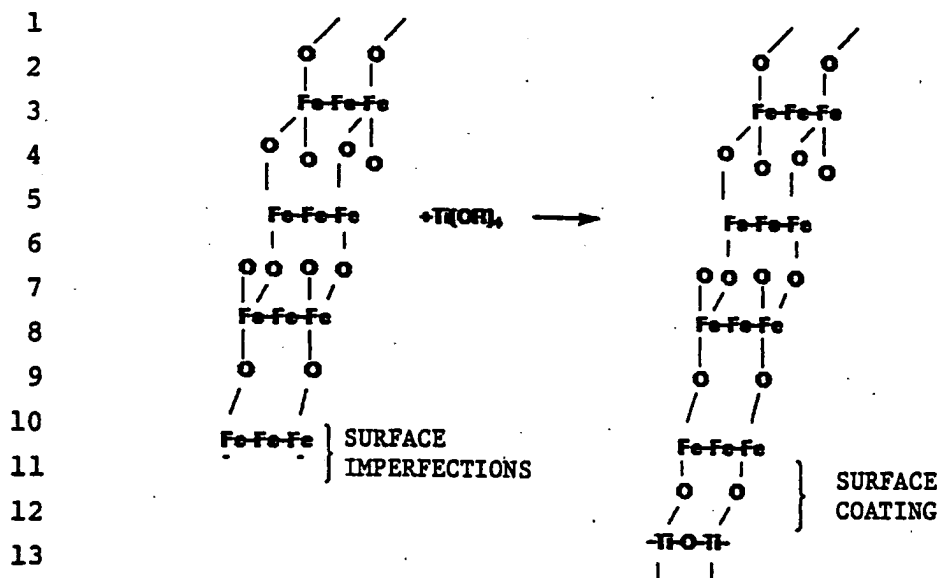
12 Several different cubic ferrites have been prepared
13 with several different crystal sizes each. The onset of
14 superparamagnetic behavior occurs at various size unit
15 crystals depending on the exact composition. Table 3 is
16 an estimate of the size where supermagnetic behavior
17 begins for several different crystal compositions.

18 TABLE 3

19	MINIMUM SIZE FOR	
20	<u>CRYSTAL COMPOSITION</u>	<u>SUPERPARAMAGNETIZATION</u>
21	Fe ₃ O ₄	50A
22	Fe _{2.5} Zn _{0.5} O ₄	80A
23	Fe ₂ ZnO ₄	120A
24	Fe _{2.5} Mn _{0.5} O ₄	100A
25	Fe ₂ MnO ₄	50A
26	Fe ₂ Sr _{0.25} Al _{0.5} O ₄	20A

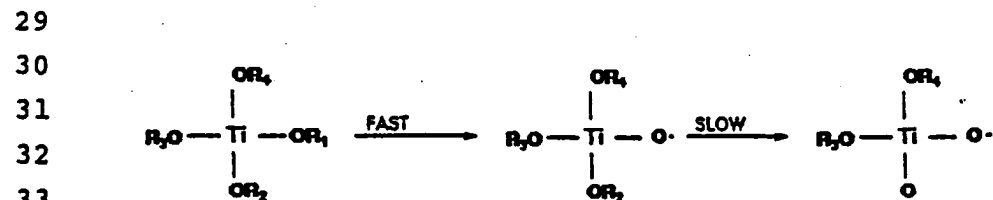
27 The substantially uniform size Fe₃O₄ affords a crystal
28 lattice which contains primarily trivalent iron (Fe⁺³) at
29 or near the surface of the crystal. It has been found
30 that these "surface trivalent" elements of the lattice
31 contain imperfections which make them available for direct
32 covalent attachment of the organo-metallic compounds of
33 the formula Ti(OR)₄ according to the following general
34 equation:

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15 It should be noted that the imperfections of the surface
16 trivalent iron is somewhat short-lived, and if organo-
17 metallic coating is delayed, oxidation can occur causing
18 the development of surface hydroxyls, which can hydrolyze,
19 to provide an FeO coating, precluding direct covalent
20 attachment of the organo-metallic moiety. For example,
21 freshly made Fe_3O_4 will spontaneously react; Fe_3O_4
22 material after 24 hours reacts but requires about 1 hour
23 of dwell time; after 48 hours the coupling reaction takes
24 place very slowly and is generally incomplete.

25 Organo-metallic compounds are preferably of the
26 formula $Ti(OR)_4$ wherein R is an alkyl group and the
27 dissociation to the reactive component follows the
28 following general reaction criterion:



35 Accordingly, R_1 , R_2 , R_3 and R_4 are selected so that rapid
36 dissociation of the first radical (R_1) is fast, and

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1 dissociation of subsequent radicals (R_2-R_4) is slow. It
2 has been found that when the radicals R_1-R_4 are
3 collectively alkyl type, the dissociation is linear with
4 respect to the length of the chain (the shorter the chain,
5 the faster the dissociation). Therefore, it is possible
6 to shift the reactivity of such organo-metallic compounds
7 by simply replacing shorter alkyl substituents with longer
8 alkyl substitution. It has also been found that when R is
9 an aryl moiety, dissociation is relatively slow. Other
10 moieties (e.g. esters, ketones) have been found to provide
11 intermediate dissociation constants.

12 Description of Chemical Bond Magneto Clusters

13 Aggregate clusters of sub 50A non-magnetic ferrites
14 were prepared by several techniques including air drying
15 of the particles to form agglomerates, argon drying at
16 room temperature, several different solution encapsulation
17 techniques and by covalent coupling of surface modified
18 crystals. All of the techniques employed provided
19 particle clusters of at least 250A diameter and mostly of
20 500A or greater. In all cases, surprisingly, the particle
21 clusters of non-magnetic ferrite crystal were magnetic.

22 Organo-metallic coating with monomer material capable
23 of adsorptive or covalent binding to iron oxide particles
24 (of less controlled particle size) is reported in U.S.
25 patent application no. 556,169, filed August 10, 1990.
26 According to the instant invention, such coatings can now
27 advantageously be applied to inorganic oxide crystal
28 particles of substantially uniform particle size
29 distribution. For example, substantially uniform sub 50A
30 Fe_3O_4 was treated with titanium tetra-isopropoxide and
31 subsequently terminated with a C-6 carboxylic acid and a
32 second population was terminated with a C-6 amine. When
33 mixed together and measured for magnetic response, no
34 magnetic moment was observed. However, upon addition of
35 methyl diisocyanate, the amine and carboxyl terminus
36 groups spontaneously caused clustered aggregates of

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1 magnetic particles to form and a magnetic moment
2 proportional to the concentration of methyl diisocyanate
3 added was observed until saturations occurred when all of
4 the amine and/or carboxyl reagent was exhausted.

5 Description of the Magnetic Molecular Switch

6 Another application for the magnetic cluster is the
7 so-called magneto-molecular switches. Sub 50A non-
8 magnetic Fe_3O_4 particles are treated by mixing them in a
9 non-aqueous solvent, such as dimethyl formamide and with
10 titanium tri-isopropoxy-3,4-dihydroxy phenoxide.

11 The particles prepared in this fashion, are titanium
12 oxide coated with o-dihydroxy benzene termination and are
13 non-magnetic in an applied field. Upon addition of a
14 solution of a transition metal, sodium molybdate and
15 tungsten, for example, a 2:1 coordination complex forms
16 between 1 metal clustered and 2 o-hydroxy benzene atoms
17 causing the particles to become clustered and giving rise
18 to a magnetic signal that is proportional to the
19 concentration of metal ion coupling formed.

20 Surprisingly, a slight change in pH causes the complex
21 to decompose and the resulting magnetization return to 0.
22 A return to the pH favorable for the formation of the
23 complex results in a renewed magnetization of equivalent
24 field strength to that achieved after initial addition of
25 metalate ion. This so called magneto-molecular switch is
26 useful for, but not limited to: magnetic tracers for in
27 vitro analysis, magnetic tracers for in vivo diagnostics,
28 magnetic processing by metals (especially for group VI
29 transition metals), analysis of metals, filtering aids,
30 magneto chromatography, and cell sorting.

31 Description of the Applications

32 The inorganic oxide crystal particles of substantially
33 uniform particle size distribution may be coupled to
34 biological or organic molecules with affinity for or the
35 ability to adsorb or which interact with certain other
36 biological or organic molecules. Particles so coupled may

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1 be used in a variety of in vitro or in vivo systems
2 involving separation steps or the directed movement of
3 coupled molecules to particular sites, including, but not
4 limited to, immunological assays, other biological assays,
5 biochemical or enzymatic reactions, affinity
6 chromatographic purifications, cell sorting and diagnostic
7 and therapeutic uses.

8 Magnetic In vitro Tracers

9 Controlled size inorganic oxide particles of this
10 invention can be covalently bonded by conventional
11 coupling chemistries to bioaffinity adsorbents including,
12 but not limited to, antibodies (ligands, e.g., anti-
13 thyroxine, anti-triiodothyronine, anti-thyroid stimulating
14 hormone, anti-thyroid binding globulin, anti-
15 thyroglobulin, anti-digoxin, anti-cortisol, anti-insulin,
16 anti-theophylline, anti-vitamin B-12, anti-folate, anti-
17 ferritin, anti-human chorionic gonadotropin, anti-follicle
18 stimulating hormone, anti-progesterone, anti-testosterone,
19 anti-estriol, anti-estradiol, anti-prolactin, anti-human
20 placental lactogen, anti-gastrin and anti-human growth
21 hormone antibodies), antigens (ligates, e.g. hormones,
22 peptides, pharmacological agents, vitamins, cofactors,
23 hematological substances, virus antigens, nucleic acids and
24 nucleotides) and specific bonding proteins, which coupled
25 particles can be used in immunoassays or other binding
26 assays for the measurement of analytes in solution. In
27 broad aspect, when such controlled size inorganic oxide
28 particles are non-magnetic, and bound to a given species
29 having specific affinity for a corresponding biochemical
30 moiety, the magnetic response becomes directly
31 proportional to the concentration of the biochemical
32 moiety causing the complexation.

33 For example, crystals are prepared that are, as
34 explained earlier, below the critical size for the
35 development of superparamagnetic behavior. The non-
36 magnetic crystals are then coated with an organo-metallic

1 coating, for example, amino-hexyl-titanium-tri-
2 isopropoxide, and thermally crosslinked to form an organo-
3 titanium polymer coating having an organic spacer arm (the
4 hexyl moiety) and organic functional group (i.e., the
5 amino-group). Anti-T-4 (thyroid hormone) with carboxylic
6 acid terminal functionality is then coupled to the non-
7 magnetic crystal in the presence of CDI (carbodiimide
8 catalyst) thereby forming an amide linkage between Anti-T-
9 4 and the coated particle. Upon the addition of T-4
10 hormone, clusters are formed, and magnetic properties are
11 detected.

12 In a further embodiment, an antibody, such as IgG, is
13 coupled to the non-magnetic crystals, followed by addition
14 of antithiophillene. Upon addition of thiophillene,
15 magnetic clusters are formed.

16 In vivo Tracers

17 A surface modification is put on the surface of non-
18 magnetic Fe_3O_4 . The modified reagent is injected into a
19 patient and a complex is formed at a specific site in the
20 body. The patient is imaged by MRI, or other suitable
21 magnetic detection techniques.

22 Magnetic Metal Processing/Metal Analysis

23 Non-magnetic Fe_3O_4 is coupled to chelating agents and
24 put into contact with the process stream. The complex
25 forms and gives rise to a magnetic moment on the cluster
26 thus formed. The cluster and metal of choice are
27 collected with a magnet. The pH is changed to strip the
28 metal and the product is collected. For example, the non-
29 magnetic crystals are prepared as described above, with an
30 organo-titanium polymer coating having an organic spacer
31 arm and a terminal amino functionality. The particles are
32 then reacted, by and through the amino functionality, with
33 2,3-dihydroxy-5-benzoic acid (upon addition of CDI) to
34 form an amide coupled product with 2,3-dihydroxy-benzene
35 termination. When such dihydroxy functionality is brought
36 into contact with metals such as Tu, or Mo, under

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1 controlled pH (6-8) a complex forms and gives rise to the
2 magnetic moment. In a similar manner, 2,3-dithio-
3 5-benzoic acid can be employed, providing terminal dithio
4 functionality, for more selective chelating with, e.g.,
5 Mo.

6 EXAMPLES

7 Example 1

8 PREPARATION OF 25A DIALYZED IRON OXIDE CRYSTAL

9 A stock of solution of iron salt is prepared by first
10 dissolving 2.5g $\text{FeCl}_2 \cdot \text{H}_2\text{O}$ (Aldrich) in 37.5g of tap water
11 at 65°C, then adding 4g FeCl_3 (Aldrich) to the solution
12 and mixing until dissolved. The solution is dark orange
13 in color. From this stock solution a dilute solution is
14 prepared for dialysis by adding 3g of the stock iron
15 solution to 297g of warm (50°C) water. 50g of this 1%
16 solution is sealed in cellulose dialysis tubing (Sigma
17 MW12000) that has been prepared in the following manner:
18 A 12 inch strip of tubing is soaked in warm water for 30
19 minutes, rinsed thoroughly in warm water and stored in
20 cool water until the addition of iron solution.

21 The dialysis tubing containing 50 g of the 1% iron
22 solution is sealed and then placed in a 2% ammonium
23 hydroxide solution:

24 6g NH_4OH (Ashland Chemical 28-30%) in 294g cool water

25 The container holding the NH_4OH solution and dialysis
26 sack of iron solution is covered tightly and allowed to
27 dialyze at room temperature until equilibrium is reached
28 (4-6 hours). An orange precipitate of iron oxide forms
29 inside the dialysis sack, white precipitate of ammonium
30 chloride forms outside the sack. The precipitate is
31 decanted from the tubing and washed by centrifuging,
32 decanting the supernatant, and adding water. This step is
33 repeated three times.

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Example 2PREPARATION OF 50A DIALYZED IRON OXIDE CRYSTAL

A stock of solution of iron salt is prepared by first dissolving 2.5g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Aldrich) in 37.5g of tap water at 65°C, then adding 4g FeCl_3 (Aldrich) to the solution and mixing until dissolved. The solution is dark orange in color. From this stock solution a dilute solution is prepared for dialysis by adding 6g of the stock iron solution to 295g of warm (50°C) water. 50g of this 2% solution is sealed in cellulose dialysis tubing (Sigma MW12000) that has been prepared in the following manner: A 12 inch strip of tubing is soaked in warm water for 30 minutes, rinsed thoroughly in warm water and stored in cool water until the addition of iron solution.

The dialysis tubing containing 50g of the 2% iron solution is sealed and then placed in a 4% ammonium hydroxide solution:

12g NH_4OH (Ashland Chemical 28-30%) in 288g cool water

The container holding the NH_4OH solution and dialysis sack of iron solution is covered tightly and allowed to dialyze at room temperature until equilibrium is reached (4-6 hours). A dark orange precipitate of iron oxide forms inside the dialysis sack, white precipitate of ammonium chloride forms outside the sack. The precipitate is decanted from the tubing and washed by centrifuging, decanting the supernatant, and adding water. This step is repeated three times.

Example 3PREPARATION OF 75A DIALYZED IRON OXIDE CRYSTAL

A stock solution of iron salt is prepared by first dissolving 2.5g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ (Aldrich) in 37.5g of tap water at 65°C, then adding 4g FeCl_3 (Aldrich) to the solution and mixing until dissolved. The solution is dark orange in color. From this stock solution a dilute solution is prepared for dialysis by adding 9g of the stock iron

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1 solution to 291g of warm (50°C) water. 50g of this 3%
2 solution is sealed in cellulose dialysis tubing (Sigma
3 MW12000) that has been prepared in the following manner:
4 A 12 inch strip of tubing is soaked in warm water for 30
5 minutes, rinsed thoroughly in warm water and stored in
6 cool water until the addition of iron solution.

7 The dialysis tubing containing 50g of the 3% iron
8 solution is sealed and then placed in a 4% ammonium
9 hydroxide solution:

10 12g NH_4OH (Ashland Chemical 28-30%) in 288g cool water

11 The container holding the NH_4OH solution and dialysis
12 sack of iron solution is covered tightly and allowed to
13 dialyze at room temperature until equilibrium is reached
14 (4-6 hours). A brown precipitate of iron oxide forms
15 inside the dialysis sack, while precipitate of ammonium
16 chloride forms outside the sack. The precipitate is
17 decanted from the tubing and washed by centrifuging,
18 decanting the supernatant, and adding water. This step is
19 repeated three times.

20

Example 4

SYNTHESIS OF TITANIUM COATED 100A MAGNETIC PARTICLES

22 Titanium coated magnetite, Fe_3O_4 , is prepared using
23 the following method:

24 Iron salts, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and FeCl_3 (41g) are each
25 dissolved in 1000 cc of water. The solutions are combined
26 into a 2 liter beaker and 70 ml of ammonium hydroxide is
27 added while mixing. The beaker containing the resulting
28 precipitate, 28 gm of Fe_3O_4 , is then placed onto a
29 permanent magnet to magnetically separate the magnetic
30 particle from the salt by-products. After resting on the
31 magnet for 5 minutes, the clear salt solution is decanted.
32 The precipitate is then resuspended in a total of 1500 cc
33 of water and placed on a permanent magnet for 5 minutes
34 before decanting. The above washing process is repeated
35 three additional times. After the final decanting, the

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1 magnetite is suspended in 1500 cc of dry acetone and
2 magnetically separated as above. The particles are
3 acetone washed a total of 3 times. After the final
4 decanting, the particles are suspended in 500 cc of N,N
5 dimethyl formamide.

6 The solution, 250 cc, is poured into a horizontal bead
7 motor mill and milled for 10 minutes to ensure efficient
8 dispersion. Titanium isopropoxide, 35 gm, dissolved in 50
9 cc of N,N dimethyl formamide is slowly pipetted into the
10 funnel of the operating motormill and milled for 15
11 minutes.

12 The dispersion is removed from the mill, magnetically
13 separated, decanted and water washed 5 times with 1000 cc
14 of distilled water.

15 Example 5

16 SYNTHESIS OF TITANIUM COATED 20A NON MAGNETIC PARTICLES

17 This example illustrates the preparation of
18 organometallic, titanium isopropoxide, coated non-magnetic
19 20A ferrites. A dispersion of non-magnetic 20A particles
20 is water washed five times and anhydrous methanol washed
21 three times by centrifugation. A total of 5.0 g of
22 particle is suspended in 250 ml of N,N dimethyl formamide
23 and milled in a bead motormill for 15 minutes. 12.0g
24 titanium isopropoxide dispersed in 30.0 g N,N- dimethyl
25 formamide is slowly pipetted into the operating mill and
26 milled for another 15 minutes. The product is then
27 removed to form the mill and water washed five times by
28 centrifugation and resuspended in distilled water.

29 Example 6

30 SYNTHESIS OF AMINE TERMINATED MAGNETIC PARTICLES

31 Magnetite coated with an organometallic, Ti, and
32 terminated with a C-6 amine is prepared using the
33 following method.

34 The precipitation, washing and coating with
35 organometallic, titanium isopropoxide, is conducted in the
36 exact manner as described above. After the washed

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1 magnetite particle, N,N- dimethyl formamide and titanium
2 isopropoxide have milled for 15 minutes, 15 gm of 6-amino
3 1-hexanol dissolved in 30 cc of N,N dimethyl formamide is
4 pipetted into the operating mill. After milling for 15
5 minutes, the dispersion is heated for 20 minutes at 100°C
6 with occasional mixing. The dispersion is then allowed to
7 cool, magnetically separated and washed five times with
8 1,000cc of distilled water.

9 Example 7

10 SYNTHESIS OF CARBOXYL TERMINATED MAGNETIC PARTICLES

11 Magnetite coated with an organometallic, Ti, and
12 terminated with a C₆ carboxyl group is prepared as
13 follows:

14 14.2 g of 4-hydroxy butyric acid sodium salt dispersed
15 in 30 cc of N,N-dimethyl formamide is slowly pipetted to
16 the 250 cc of washed organometallic coated magnetic
17 particles as described above in Example 4. After milling
18 for 15 minutes, the dispersion is heated for 20 minutes
19 at 100°C with mixing. The solution, at room temperature,
20 is magnetically separated and washed five times with 1,000
21 cc of distilled water.

22 Example 8

23 SYNTHESIS OF DIHYDROXY AROMATIC TERMINATED MAGNETIC PARTICLES

24 This example illustrates the preparation of dihydroxy-
25 aromatic terminated magnetic particle. 5 g of magnetite
26 coated with titanium isopropoxide and 6-amino-1-hexanol,
27 prepared as above, is dispersed in sodium metabisulfite
28 and distilled water solution, 300 cc. The sodium
29 metabisulfite solution has been pretreated with nitrogen
30 gas to prevent oxidation of the particles. 78 g of gallic
31 acid, and 1.0 g of carbodimide is combined with the
32 amine-terminated magnetic particle with mixing. After
33 incubating for one hour, the product is magnetically
34 separated and water washed.

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Example 9MAGNETIC TRACERS FOR IMMUNO ASSAY I

20A non-magnetic ferrite particles were washed 4 times with water, 3 times with acetone and 3 times with anhydrous methanol by collecting the particles after centrifugation and resuspending the particles by vigorous agitation.

Tyzor (titanium tetra-isopropoxide), dissolved in anhydrous methanol was added to 0.53 g dry of particles at 25 g Ti/9.6 g dry particles. Steel balls were added and the particles were milled in a ball mill for one hour.

The particles were then amine terminated by adding 6-amino-1-hexanol dissolved in anhydrous methanol to the Tyzor coated particles. For every 9.6 g dry particles, .088 mol amine was used. This was added to the Tyzor coated particles and milled on the ball mill for 3 1/2 hours. The magnetics were tested on a vibrating sample magnetometer. The particles were found to be non-magnetic.

The sample was divided into 4 equal dry parts of 0.13 g each. 1,6 diisocyanato-hexane was added to particles in four concentrations: 0, .5, 4, 8 lm 1,6 diiso./.5 g dry. The particles were milled overnight in the ball mill without using steel balls.

The magnetics were tested again on the VSM. It was determined that the increase in 1,6 diisocyananatohexane resulted in a proportional increase in magnetivity.

Example 10ENCAPSULATION BY A POLYMER

20A non-magnetic ferrite particles were washed 4 times with water, 5 times with acetone, (collecting with a centrifuge between washes). The acetone slurry is then washed 5 times with hexane. A solvent borne solution of the polymer (e.g., polystyrene, polyurethane, poly(vinyl chloride)) from about 0.1%-10% by weight in an amount equal to about 1:10 to 10:1 particle:polymer ratio is then

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1 added. Mixing continues for about 10 minutes in a high
2 shear mixer to allow the crystals to coat uniformly with
3 polymer. Water is then added in a volume equal to about
4 10-100 times the amount of solvent to flocculate the
5 polymer. The beads are then collected. In the case of
6 polyurethane, it has been found the THF is the solvent of
7 choice.

8

Example 11

9

ADDITION OF MONOMER FOLLOWED BY CROSSLINKING

10 A particle slurry is prepared as in Example 10. Oleic
11 acid is then added to the hexane slurry of particles and
12 mixed in a high shear mixer for about 20 minutes. A
13 volume of acetone is then added, equal to approximately 5
14 times the amount of hexane to the oleic acid coated
15 particle dispersion, in order to flocculate. The
16 resulting residue is collected and mixed in water in a
17 high shear mixer for about 1 hour to produce oleic acid
18 coated crystal beads. The bead slurry is then exposed to
19 3-beam generator (Energy sources, Woburn, MA), from 1-20
20 meg Rad for about 0.25-0.5 sec., to crosslink through the
21 unsaturated group.

22

Example 12

23

PREPARATION OF SUB 10 NM PARTICLES IN A TWO-SIDED

24

DIALYSIS TANK

25 2 nm diameter uniform magnetic crystals were prepared
26 by controlled contact of a base solution and iron salt
27 solution across a semipermeable membrane, resulting in an
28 iron oxide crystal precipitate of defined size within a
29 narrow size distribution range. A Spectra/Por® 5 dialysis
30 membrane (flat sheet) was affixed in a manner as to
31 separate two equal sized chambers of a two sided Dialysis
32 reaction tank. Both sides of the tank were filled with 20
33 liters of distilled H₂O at 20°C. 12.5g FeCl₂ 4H₂O and 20g
34 FeCl₃ were added to one chamber of the tank and stirred
35 until dissolved. 60.6g NaOH were dissolved in 2 liters of
36 H₂O and added to the solution into the opposite chamber in

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1 the tank. Both sides were agitated by a mechanical paddle
2 stirrer for 15 min. After 70-80 hours of contact time,
3 the iron solution and precipitated crystals were removed
4 from the tank and the magnetic crystals were collected by
5 centrifugation and measures by TEM to be 2 nm average
6 diameter.

7 Uniform size inorganic core particles can be prepared
8 by the preferred method reported in U.S. Patent
9 Application Serial No. 894,260, filed June 8, 1992, the
10 teachings of which are incorporated by reference. As
11 described therein, aqueous solutions of an inorganic salt
12 and an inorganic base are contacted across a porous
13 membrane wherein the membrane contains a plurality of
14 pores which allows for precipitation of substantially
15 monodispersed inorganic oxide particles on one side of the
16 membrane and precipitation of a salt of the corresponding
17 base on a second side of the membrane. Particle size
18 diameter can range between 5-1000 Angstroms, and in a
19 preferred embodiment, 5-100 Angstroms, with a particle
20 size distribution of +/- 10%. The inorganic salts are of
21 the formula MY, wherein M is selected from the group
22 consisting of Fe, Co, Ni, Zn, Mn, Mg, Ca, Ba, Sr, Cd, Hg,
23 Al, B, Sc, Ga, V, In, and mixtures thereof, with Y being
24 selected from the group consisting of Cl, Br, I, SO₄, NO₃,
25 PO₄ and mixtures thereof. The inorganic base is selected
26 from the group consisting of NH₄OH, KOH, LiOH, NaOH, CsOH,
27 RbOH and mixtures thereof. Accordingly, and in a
28 preferred embodiment, Fe₃O₄ is prepared (a mixed
29 Fe(+2)Fe(+3) oxide of the formula [Fe(+2)][Fe(+3)]₂O₄)
30 with a uniform sub 100 Angstroms diameter serving as the
31 inorganic core of the liposomes described herein.

32 Inorganic core particles can also be prepared
33 according to the following general procedure: metal
34 salts, or organometallocenes are precipitated in base at
35 high temperature and pressure to form fine magnetic metal
36 oxide crystals. The crystals are redispersed, then washed

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1 in water and an electrolyte. Magnetic separation can be
2 used to collect the crystal between washes. The crystals
3 are then milled to a more controlled particle size, for
4 example, in a ball mill, under conditions sufficient to
5 form 50 Angstroms or lower particle size. See, U.S.
6 Patent No. 5,071,076, and U.S. Patent Application Serial
7 No. 806,478, filed December 31, 1991, the teachings of
8 which are incorporated by reference.

9 III. Amphipathic Organic Compounds

10 The amphipathic organic compounds which can be
11 used in forming the inorganic core liposome of the
12 invention may be selected from a variety of organic
13 compounds which contain both a hydrophobic and hydrophilic
14 moiety. According to one important aspect of the
15 invention, it has been discovered that the hydrophilic
16 moiety is adsorbed or coordinated onto the surface of the
17 inorganic oxide, whereas the hydrophobic moiety of the
18 molecule extends outwardly to associate with the
19 amphipathic vesicle forming lipid compounds. Preferred
20 amphipathic organic compounds include fatty acids selected
21 from the group consisting of oleic, stearic, linoleic,
22 lionlenic, palmitic, nyristic and arachidonic acid.

23 IV. Amphipathic Vesicle Forming Lipid Components

24 The lipid components used in forming the
25 inorganic core liposomes of the invention may be selected
26 from a variety of vesicle forming lipids, typically
27 including phospholipids, such as phosphatidylcholine (PC),
28 phosphatidic (PA), phosphatidylinositol (PI),
29 sphinogomyelin (SM), and the glycolipids, such as
30 cerebroside and gangliosides. The selection of lipids is
31 guided by consideration of (a) drug release rate is serum,
32 (b) drug-entrapment efficiency, (c) liposome toxicity, and
33 (d) biodistribution and targeting properties. A variety
34 of lipids having selected chain compositions are
35 commercially available or may be obtained by standard

-55-

1 lipid isolation procedures. See, e.g. U.S. Patent No.
2 4,994,213.

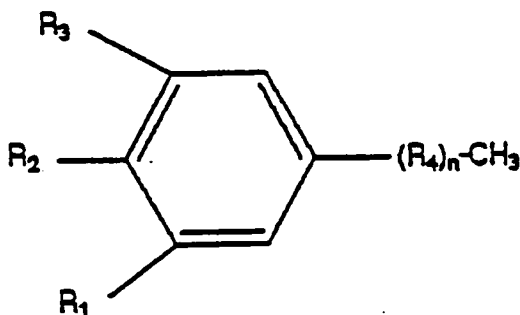
3 The lipids may be either fluidic lipids, e.g.
4 phospholipids whose acyl chains are relatively
5 unsaturated, or more rigidifying membrane lipids, such as
6 highly saturated phospholipids. Accordingly, the vesicle
7 forming lipids may also be selected to achieve a selected
8 degree of fluidity or rigidity to control the stability of
9 the liposome in serum and the rate of release of entrapped
10 drug from the liposome in the bloodstream. See, e.g. U.S.
11 Pat. No. 5,013,556.

12 In a preferred embodiment, the vesicle forming lipid
13 include those phospholipids in which the polar-head-group
14 region is modified by the covalent attachment of
15 polyalkylene ether polymers of various molecular weights.
16 The attachment of the relatively hydrophilic polyalkylene
17 ether polymer, particularly polyethylene oxide, alters the
18 hydrophilic to hydrophobic balance within the phospholipid
19 in order to give unique solubility to the phospholipid
20 compound in an aqueous environment. See, e.g. U.S. Pat.
21 No. 4,426,330. The polyalkyl ether lipid is preferably
22 employed in the inorganic core liposome composition in an
23 amount between about 1-20 mole percent, on the basis of
24 moles of derivatized lipid as a percentage of total moles
25 of vesicle-forming lipids. The polyalkylether moiety of
26 the lipid preferably has a molecular weight between about
27 120-20,000 daltons, and more preferably between about
28 1000-5000 daltons.

29 In yet another embodiment of the present invention, a
30 new series of phenyl lipid compounds are described which

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1 have the following structural formula:



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11 wherein two of R_1 , R_2 and R_3 represent a saturated or
12 unsaturated straight-chain or branched chain hydrocarbon
13 group, the other being hydrogen, therein providing at
14 least two hydrocarbon chains attached to the phenyl
15 moiety, wherein the two hydrocarbon chains are typically
16 between about 14-22 carbon atoms in length, and have
17 varying degrees of unsaturation. R_4 represents the
18 repeating unit of either a poly(alkylene oxide) polymer,
19 preferably ethylene, propylene and mixtures thereof, or
20 the repeating unit of poly(vinyl alcohol). The number of
21 alkylene oxide or vinyl alcohol groups in the polymer,
22 designated as n , may vary from 0 to about 200 or more.

23

24 V. Preparing the Inorganic Core Liposome Composition

25 One preferred method for producing the uniform
26 size inorganic core liposome composition begins with first
27 coating the magnetic particles described above in Section

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1 II with an amphipathic organic compound which contains
2 both a hydrophilic and hydrophobic moiety. For example,
3 fatty acids, such as oleic acid, linoleic acid or
4 linolenic acid, dispersed in an organic solvent, are
5 directly added to the particles at a ratio of dry
6 Fe_3O_4 :acid equal to 2:1 weight percent. After
7 mechanically milling the mixture for 1 to 1.5 hours on a
8 ball mill with 4 mm glass media, the acid coated particles
9 collapse around the media allowing for easy removal of
10 water without the loss of the particles. The coated
11 particles are then dispersed in an organic solvent by
12 addition of 700 ml of hexane, toluene or chloroform and
13 mechanically milling with glass media overnight (15 hrs).

14 Absorbing a phospholipid onto the fatty acid coated
15 particles was accomplished by addition of a synthetic
16 polyethylene glycol terminated phosphatidyl ethanolamine
17 to the above dispersion and mechanically mixing for 3
18 hours. The ratio of fatty acid:pure lipid is about 1:1
19 weight percent.

20 To transfer the particles from an organic phase to an
21 aqueous phase, 7 mls of the dispersion was placed into a
22 14 ml glass vial with 3 ml of distilled water. The vial
23 was placed in warm, 35°C sonicating water bath with N_2
24 bubbling through it to evaporate the solvent. Once the
25 solvent has evaporated, the aqueous dispersion was then
26 suspended in a total of 10 mls of autoclaved water,
27 sonicated for one hour, and centrifuged for 5 minutes.
28 The supernatant was removed and brought to 20 mg
29 particle/ml solution with autoclaved water.

30 VI. Utility

31 From the above, it can be appreciated that the
32 present invention offers a number of advantages over prior
33 art liposome-methods. The preparation of uniform size
34 inorganic core particles by dialysis and precipitation
35 across a semi-permeable membrane is unique in its ability
36 to allow for the production of uniform size liposomes

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1 without the requirement for extrusion or other additional
2 liposome sizing techniques. The ability to selectively
3 vary the average size of liposomes, according to lipid
4 composition and/or ionic strength, is another useful
5 feature of the invention. While the present invention
6 provides inorganic core liposomes with a size range of
7 about 5-5000 nm, one selected size range, between about
8 100-300 nm, is particularly useful for a variety of
9 parenteral uses, as discussed.

10 One general class of drugs include water-soluble
11 liposome permeable compounds which are characterized by a
12 tendency to partition preferentially into the aqueous
13 compartments of the liposome suspension, and to
14 equilibrate, over time, between the inner liposomal spaces
15 and outer bulk phase of the suspension. Representative
16 drugs in this class include terbutaline, albuterol,
17 stropine methyl nitrate, cromolyn sodium, propracalol,
18 funoisolide, ibuprofen, geniamycin, tobermycin,
19 pentamidine, penicillin, theophylline, bleomycin,
20 etopoxide, captoprel, n-acetyl cystein, verapamil,
21 vitamins, and radio-opaque and particle-emitter agents,
22 such as chelated metals. Because of the tendency of these
23 agents to equilibrate with the aqueous composition of the
24 medium, it is preferred to store the liposome composition
25 in lyophilized form, with rehydration shortly before
26 administration.

27 A second general class of drugs are those which are
28 water-soluble, but liposome-impermeable. For the most
29 part, these are peptide or protein molecules, such as
30 peptide hormones, enzymes, enzyme inhibitors,
31 apolipoproteins, and higher molecular weight carbohydrates
32 characterized by long-term stability of encapsulation.
33 Representative compounds in this class include calcitonin,
34 atriopentin, -1 antitrypsin (protease inhibitor),
35 interferon, oxytocin, vasopressin, insulin, interleukin-2,
36 superoxide dismutase, tissue plasminogen activator (TPA),

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1 plasma factor 8, epidermal growth factor, tumor necrosis
2 factor, lung surfactant protein, interferon, lipocortin,
3 α -interferon, macrophage colony stimulating factor, and
4 erythropoietin.

5 A third class of drugs are lipophilic molecules. The
6 drugs in this class are defined by an oil/water partition
7 coefficient, as measured in a standard oil/water mixture
8 such as octanol/water, of greater than 1 and preferably
9 greater than about 5. Representative drugs include
10 prostaglandins, amphotericin B, progesterone, isosorbide
11 dinitrate, testosterone, nitroglycerin, estradiol,
12 doxorubicin, epirubicin, beclomethasone and esters,
13 vitamin E, cortisone, dexamethasone and esters, and
14 betamethasone valerate.

15 In another application, the inorganic core liposome
16 composition is designed for targeting a specific target
17 tissue or organ. For example, this feature allows for
18 targeting a tumor tissue, for drug treatment by
19 intravenous administration to a tumor-bearing subject.

20 As another example, the inorganic core liposomes may
21 be prepared with surface-bound ligand molecules, such as
22 antibodies, which are effective to bind specifically and
23 with high affinity to ligand-binding molecules such as
24 antigens, which are localized specifically on target
25 cells.

26 A variety of methods for coupling ligands to the
27 surface of liposomes are known, including the
28 incorporation of ligand-derivatized lipid components into
29 liposomes or coupling of ligands to activated liposome
30 surface components.

31 The targeted inorganic core liposomes may be prepared
32 to include cancer chemotherapeutic agents, such as those
33 listed above. In one preferred embodiment, the liposomes
34 are prepared to include PEG-PE and PG, to a final

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1 concentration of charged lipids up to 40 mole percent,
2 doxorubicin, and remainder neutral phospholipids or
3 neutral phospholipids and cholesterol.

4 In an inorganic core liposome composition which is
5 useful for radio-imaging of solid tumor regions, the
6 liposomes are prepared with encapsulated radio-opaque or
7 particle-emission metal, typically in a chelated form
8 which substantially prevents a permeation through the
9 liposome bilayer.

10 In still another application, the liposome composition
11 is designed to enhance uptake of circulating cells or
12 other blood-borne particles, such as bacteria, virus-
13 infected blood cells and the like. Here the long-life
14 liposomes are prepared to include surface-bound ligand
15 molecules, as above, which bind specifically and with high
16 affinity to the selected blood-borne cells. Once bound to
17 the blood-borne particles, the liposomes can enhance
18 uptake by the RES.

19 Polyalkylether moieties on the liposomes may be
20 derivatized by the associated amphipathic lipid by an
21 ester, peptide, or disulfide bond which can be cleaved,
22 after liposome binding, to the target cells, to further
23 enhance RES particle clearance.

24 Studies performed in support of the present invention
25 indicate that the inorganic core liposome composition of
26 the invention provides an enhancement in blood circulation
27 lifetime which is equal, and in some cases superior, to
28 the most effective RES-evading rigid-lipid liposomes which
29 have been reported heretofore, including liposomes
30 containing GMI and membrane-rigidifying lipids.

31 The blood circulation lifetimes achieved in the
32 present invention should be substantially greater than
33 with fluid-core liposomes.

34 The following examples illustrate methods of
35 preparation of inorganic core liposomes with enhanced
36 circulation times, and for accessing circulation times in

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1 vivo and invitro. The examples are intended to illustrate
2 specific inorganic-core liposome compositions and methods
3 of the invention, but are in no way intended to limit the
4 scope thereof.

5 DESCRIPTION OF THE EMBODIMENTS

6 EXAMPLE 1

7 Preparation of Magnetic Particles by Co-precipitation
8 of Fe+2/Fe+3 with Excess Base

9 Magnetic particles of 100 Angstroms in diameter are
10 prepared using the following method. Iron salts, FeCl_2^- ,
11 $3\text{H}_2\text{O}$, (25g), and FeCl_3 (41g) are each dissolved in 1000 cc
12 of water. The solutions are combined into a 2 liter
13 beaker and 70ml of ammonium hydroxide is added while
14 mixing. The resulting black magnetic precipitate yields
15 28gm of magnetite, Fe_3O_4 .

16 EXAMPLE 2

17 Preparation of sub 10 nm particles

18 2 nm diameter uniform magnetic crystals were prepared
19 by controlled contact of a base solution and iron salt
20 solution across a semipermeable membrane, resulting in an
21 iron oxide crystal precipitate of defined size within a
22 narrow size distribution range.

23 A Spectra/Por 5 dialysis membrane (flat sheet) was
24 affixed in a manner as to separate two equal sized
25 chambers of a two sided Dialysis reaction tank. Both
26 sides of the tank were filled with 20 liters of distilled
27 H_2O at 20°C . 12.5 g $\text{FeCl}_2^- \cdot 4\text{H}_2\text{O}$ and 20g FeCl_3 were added
28 to one chamber of the tank and stirred until dissolved.
29 60.6g NaOH were dissolved in 2 liters of H_2O and added to
30 the solution into the opposite chamber in the tank. Both
31 sides were agitated by a mechanical paddle stirrer for 15
32 min. After 70-80 hours of contact time, the iron solution
33 and precipitated crystals were removed from the tank and
34 the magnetic crystals were collected by centrifugation and
35 measures by TEM to be 2nm average diameter.

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EXAMPLE 3

Preparation of Oleic Acid Coated Magnetite

Magnetic particles, Fe_3O_4 , coated with oleic acid are prepared using magnetite as precipitated in Example 1. The magnetite is water washed by successive additions of distilled water to a slurry concentrate of magnetite. The beaker containing the magnetite slurry is placed onto a permanent magnet to magnetically separate the magnetic particle from the salt by-products between each successive addition of water. After resting the slurry on the magnet for 5 minutes, the aqueous salt solution is decanted. The precipitate is then resuspended with agitation in a total of 1500 cc of water and placed on a permanent magnet for 5 minutes before decanting. The above washing process is repeated three additional times with water. After the final water wash is decanted, the particles are acetone washed and hexane washed a total of 5 times each in the above manner.

Oleic acid is added to the magnetic hexane slurry in a ratio of oleic acid:dry particle equal to 2:1 weight percent. The mixture is adjusted to 15% total solids with hexane and mechanically milled overnight in a glass jar half filled with 3mm stainless steel media.

EXAMPLE 4

Preparation of Oleic Acid Coated Dialyzed

Magnetic Particles

Dialyzed particles coated with oleic acid are prepared using particles as prepared in Example 2. 0.1 grams of particles are washed with three 200 ml volumes of distilled water and acetone by suspending approximately 0.1gm dry particle in 200 ml of acetone and centrifuging for 45 minutes to collect particles between each washing.

Oleic acid was added to the acetone slurry in a ratio of oleic acid:dry particle equal to 2:1 weight percent and mechanically milled overnight in a glass jar half filled with 3mm glass media.

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EXAMPLE 5

Preparation of Magnetite Core Liposomes using
Phosphatidyl Choline

10 gms Oleic acid coated magnetite as prepared in Example 3 was dispersed in 100 cc hexane. The phosphate lipid is absorbed onto the particle by dissolving phosphatidyl choline (Sigma, P-3644, L-2, lecithin, 45% PC) into hexane with heating to create a 15% solution. The PC/hexane solution is combined with the magnetic/hexane solution at a ratio of pure phosphatidyl choline:oleic acid equal to 1:2 weight percent.

The solution was mixed in a glass jar (without media) on a jar roller for two hours. After mixing, the lipid was absorbed onto the particle by adding three times as much acetone than hexane and collecting the lipid coated particles over a magnet. After the coated magnetic particles were separated from the solvents, the solvents were decanted, distilled water was added to produce a 2.0% TS slurry. The slurry is heated in a beaker on a hot plate to 100°C for 10 min. From 0.5 to 50 grams of triton x-114 (Union Carbide) was added to disperse the lipidized magnetic particles in an aqueous system. A ratio of triton x114:lipid particle equal to 1:6 weight percent was the optimum level for the dispersion. The dispersion was mixed on a laboratory vortex mixer for 2 minutes and placed in an ultrasonic bath (Branson 1200, VWR) for 1 hours. The final dispersion is adjusted to 0.2% TS (2mg/ml). Particles were measured on a Nycomp laser particle size analyzer and were found to be approximately 200 nm in diameter.

EXAMPLE 6

Preparation of Phenyl Lipid

A. Synthesis of a m-isophthalic acid based phenyl lipid.

The starting material for this synthesis is 5-Aminoisophthalic acid. The 5-aminoisophthalic acid is not soluble in dioxane alone. It is soluble in a mixture

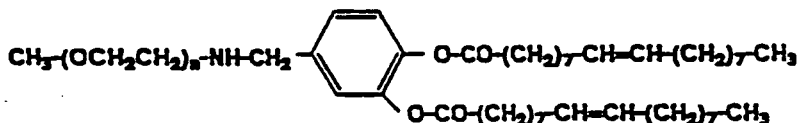
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1 of dioxane and triethylene glycol. 5-aminoisophthalic
 2 acid (145 mg) was dissolved in 5 ml. of dioxane and 2 ml.
 3 of triethylene glycol, and the pH was adjusted to 10 with
 4 NaOH. Methoxypolyoxyethylene imidazoly carbonyl, average
 5 mol. wt. 5,000 from Sigma (2.0g) was dissolved in 2ml of
 6 H₂O, 1.0ml of 1N Na₂CO₃, and 2.0 ml of triethylene
 7 glycol. This solution was added to the 5-aminoisophthalic
 8 acid solution and stirred for 36 hours at room
 9 temperature. The reaction mixture was then dialyzed
 10 overnight against 2 liters of H₂O. The dialyzed reaction
 11 mixture was mixed with 100ml of pyridine and the liquids
 12 removed via rotary evaporation. The resulting yellow oil
 13 was placed in the refrigerator. After several days a
 14 white precipitate formed. The precipitate contains both
 15 coupled and uncoupled PEG.

16 Oleyl alcohol can be coupled to the above isophthalic
 17 acid derivative using thionyl chloride. The thionyl
 18 chloride can be used to activate the oleyl alcohol for
 19 ester formation with the carboxyl groups of the
 20 isophthalate. See. Fig. 2.

21 B. Synthesis of ortho phenyl lipids

22 The ortho analog of the phenyl lips can be
 23 synthesized starting with either 3,4 dihydroxybenzaldehyde
 24 or 3,4 dihydroxybenzoic acid. The aldehyde group can be
 25 coupled to an amino group by forming the Schiff's base and
 26 then reducing it with NaBH₄. Olegic acid could then be
 27 coupled to the hydroxyl groups using thionyl chloride to
 28 provide:



34 3,4 dihydroxybenzoic acid could be coupled through
 35 its carboxyl group to amino-terminated PEG using

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1 dicyclohexyl carbodiimide. Oleic acid could then be
2 coupled as above.

3 Since both amino and carboxyl PEG derivatives as well
4 as both oleic acid and oleylamine are available, the PEG
5 and oleic acid groups can be easily interchanged in the
6 above compounds.

7 VII. Preparation of Wave Absorbing Magnetic Core
8 Particles

9
10 The wave absorbing magnetic core particles suitable in
11 the present invention are those particles which, upon
12 application of an electromagnetic field, create inductive
13 heat local to the particle. In a preferred embodiment,
14 the wave absorbing magnetic core particles comprise
15 ferrite or mixed ferrite materials, preferably of a
16 uniform, controllable size, and narrow size distribution,
17 wherein the primary component, the oxide, is of the
18 formula $M_2(+3)M(+2)O_4$, wherein M(+3) is Al, Cr or Fe, and
19 M(+2) is Fe, Ni, Co, Zn, Zr, Ca, Ba, Mg, Ga, Gd, Mn or Cd.
20 In a further aspect, the oxides can be advantageously
21 mixed with LiO, NaO and KO, or with α or γ Fe_2O_3 and
22 Fe_3O_4 .

23 The preparation of substantially uniform size oxides,
24 1 to 50,000 nm in diameter, is achieved by conversion of
25 hydrous oxide gels, in a multi-step process, wherein
26 alkali is added to individual M(+3) and M(+2) aqueous
27 solutions, which separately precipitate the corresponding
28 metal hydroxide. The two precipitates are then coarsely
29 mixed to provide micron size amorphous gel particles,
30 which can be milled to form hydrous oxide gel particles
31 about 100 Å in diameter. These particles are then heated
32 to effect dehydration, in the presence of oxygen or air,
33 wherein the dehydration temperature, time of dehydration,
34 and concentration of oxygen or air operate to control the
35 particle size of the oxide crystals therein produced.

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1 For example, in connection with the above, a
2 dehydration temperature of 100°C, at a time of about 6
3 hours, in the presence of oxygen, provides oxides
4 particles of about 70A diameter. Alternatively, a
5 dehydration temperature of about 65°C, at a time of about
6 24-36 hours, in the presence of oxygen, affords oxide
7 particle sizes of about 1000-2000A. Accordingly, by
8 recognizing that short dwell times and high temperature
9 promote small oxide particle formation, and that long
10 dwell times and low temperature promote large particle
11 formation, oxide particles from 50A to several microns in
12 diameter have been produced.

13 Heretofore, the use of ferrite materials as a
14 protective medium for electromagnetic radiation reflecting
15 surfaces was well known. In the present invention,
16 however, it has been found that very small ferrosinial
17 particles provide a high degree of absorbtion of
18 electromagnetic waves. It has also been found that the
19 complex permeability of certain ferromagnetic metallic
20 oxides varies with frequency in such a way as to provide
21 high absorption of electromagnetic magnetic radiation over
22 wide frequency ranges without using large amounts of
23 absorber material. Upon exposure to electromagnetic
24 waves, these ferrites generate significant infra-red
25 radiation over short distances local to the ferrite
26 particle's surface.

27 In general, those ferrites suitable for use in the
28 present invention are cubic crystalline materials
29 characterized by a spinal structure containing Fe_2O_3 and
30 at least one other oxide, usually of a bivalent metal,
31 e.g. lithium oxide, cadmium oxide, nickel oxide, iron
32 oxide and zinc oxide.

33 The ferrite materials of this invention can also be
34 prepared by a thermal process, in which they are mixed
35 together then ground together mixed and fired at about
36 1200°C in a tube furnace for four hours or made by

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1 oxidation of ferrite powders from metal hydroxide gels.
2 The imaginary permeability must be high enough to produce
3 a large loss. For high frequencies, it has been found
4 that nickel can replace lithium and for narrow bands zinc
5 can replace cadmium.

6 One preferred mixed ferrite having the composition
7 0.45 LiO , $0.5 \text{ Fe}_2\text{O}_3$ + $0.30 \text{ CdFe}_2\text{O}_4$ + $0.25 \text{ Fe}_3\text{O}_4$ yielded
8 the following results:

9	10 Frequency Range (mHz)	11 % Absorbance	12 Surface Temp
13	14 1800-2500	15 98	16 230

17 As noted above, ferrites of interest to this invention
18 can also be prepared by conversion of hydrous oxide gels
19 in a multi-step process. In one particular preferred
20 example, alkali is added to a ferrous sulphate solution at
21 a temperature between 15 and 40°C, in a stoichiometric
22 amount adapted to precipitate ferrous hydroxide, from the
23 Fe^{++} ion. At the conclusion of said precipitation, air is
24 blown into the slurry, thus oxidizing ferrous hydroxide to
25 goethite, $\text{FeO}(\text{OH})$.

26 During a second step, alkali is added to the slurry
27 obtained in the first step. The remaining Fe^{++} is
28 precipitated in the form of ferrous hydroxide, and the
29 slurry is heated to a temperature between 70°C and 100°C
30 thus causing the formation of ferrite which is then
31 separated from the solution.

32 Accordingly, the present invention provides a process
33 suitable for treating ferrous sulphate solutions in order
34 to obtain ferrite exhibiting an equiaxial morphology with
35 a narrow particle size distribution.

36 VIII. Amphipathic Organic Compounds

37 The amphipathic organic compounds which can be used in
38 forming a liposome composition comprising the wave

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1 absorbing magnetic core particle may be selected from a
2 variety of organic compounds which contain both a
3 hydrophobic and hydrophilic moiety. According to one
4 important aspect of the invention, it has been discovered
5 that the hydrophilic moiety is adsorbed or coordinated
6 onto the surface of the wave adsorbing magnetic core
7 particle, whereas the hydrophobic moiety of the molecule
8 extends outwardly to associate with amphipathic vesicle
9 forming lipid compounds.

10 When the wave absorbing magnetic core particle is
11 freshly made Fe_3O_4 , it has been found, as reported in U.S.
12 Patent Application Serial No. 894,260, filed June 8, 1992,
13 that surface trivalent elements of the core particle
14 contain imperfections which makes them available for
15 direct covalent attachment with organometallic compounds
16 of the formula $\text{Ti}(\text{OR})_4$, wherein R is an alkyl group.
17 Accordingly, the wave absorbing magnetic core particle can
18 be coated with an organometallic coating covalently bonded
19 to said particle wherein the bonding does not depend upon
20 hydroxy functionality on the surface of said particle.
21 Such coated particles can then be associated with an
22 amphipathic vesicle forming lipid.

23 Preferred amphipathic organic compounds include fatty
24 acids selected from the group consisting of oleic,
25 stearic, linoleic, linolenic, palmitic, myristic and
26 arachidonic acid.

27 IX. Amphipathic Vesicle Forming Lipid

28 The lipid components used in forming the wave
29 absorbing magnetic core particle liposomes of the
30 invention may be selected from a variety of vesicle
31 forming lipids, typically including phospholipids, such as
32 phosphatidylcholine (PC), phosphatidic (PA),
33 phosphatidylinositol (PI), sphingomyelin (SM), and the
34 glycolipids, such as cerebroside and gangliosides. The
35 selection of lipids is guided by consideration of liposome
36 toxicity and biodistribution and targeting properties. A

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1 variety of lipids having selected chain compositions are
2 commercially available or may be obtained by standard
3 lipid isolation procedures. See, e.g. U.S. Patent No.
4 4,994,213.

5 The lipids may be either fluidic lipids, e.g.
6 phospholipids whose acyl chains are relatively
7 unsaturated, or more rigidifying membrane lipids, such as
8 highly saturated phospholipids. Accordingly, the vesicle
9 forming lipids may also be selected to achieve a selected
10 degree of fluidity or rigidity to control the stability of
11 the liposome in serum. See, e.g. U.S. Pat. No. 5,013,556.

12 In a preferred embodiment, the vesicle forming lipid
13 include those phospholipids in which the polar-head-group
14 region is modified by the covalent attachment of
15 polyalkylene ether polymers of various molecular weights.
16 The attachment of the relatively hydrophilic polyalkylene
17 ether polymer, particularly polyethylene oxide, alters the
18 hydrophilic to hydrophobic balance within the phospholipid
19 in order to give unique solubility to the phospholipid
20 compound in an aqueous environment. See, e.g. U.S. Pat.
21 No. 4,426,330. The polyalkyl ether lipid is preferably
22 employed in the wave absorbing magnetic core particle
23 liposome composition in an amount between about 1-20 mole
24 percent, on the basis of moles of derivatized lipid as a
25 percentage of total moles of vesicle-forming lipids. The
26 polyalkylether moiety of the lipid preferably has a
27 molecular weight between about 120-20,000 daltons, and
28 more preferably between about 1000-5000 daltons.

29 In yet another embodiment of the present invention,
30 phenyl lipid compounds (as reported in U.S. Application
31 Serial No. 958,646) can be employed as amphipathic vesicle
32 forming lipid components. These phenyl lipids have the

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1 structural formula:

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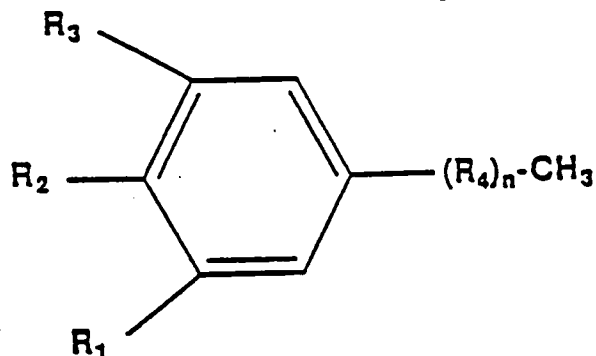
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11 wherein two of R_1 , R_2 and R_3 represent a saturated or
12 unsaturated straight-chain or branched chain hydrocarbon
13 group, the other being hydrogen, therein providing at
14 least two hydrocarbon chains attached to the phenyl
15 moiety, wherein the two hydrocarbon chains are typically
16 between about 14-22 carbon atoms in length, and have
17 varying degrees of unsaturation. R_4 represents the
18 repeating unit of either a poly(alkylene oxide) polymer,
19 preferably ethylene, propylene and mixtures thereof, or
20 the repeating unit of poly(vinyl alcohol), or a
21 polycarbohydrate. The number of alkylene oxide or vinyl
22 alcohol groups in the polymer, designated as n , may vary
23 from 0 to about 200 or more.

24

25 X. Preparing the Wave Absorbing Magnetic Core
26 Particle Liposome Composition

27 One preferred method for producing the wave absorbing
28 magnetic core liposome composition begins with first
29 coating the magnetic particles described above in Section
30 II with an amphipathic organic compound which contains
31 both a hydrophilic and hydrophobic moiety. For example,
32 fatty acids, such as oleic acid, linoleic acid or
33 linolenic acid, dispersed in an organic solvent, are
34 directly added to the particles at a ratio of dry
35 ferrite:acid equal to 2:1 weight percent. After
36 mechanically milling the mixture for 1 to 1.5 hours on a

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1 ball mill with 4 mm glass media, the acid coated particles
2 collapse around the media allowing for easy removal of
3 water without the loss of the particles. The coated
4 particles are then dispersed in an organic solvent by
5 addition of 700 ml of hexane, toluene or chloroform and
6 mechanically milling with glass media overnight (15 hrs).

7 Absorbing a phospholipid onto the fatty acid coated
8 particles was accomplished by addition of a synthetic
9 polyethylene glycol terminated phosphatidyl ethanolamine
10 to the above dispersion and mechanically mixing for 3
11 hours. The ratio of fatty acid: pure lipid is about 1:1
12 weight percent.

13 To transfer the particles from an organic phase to an
14 aqueous phase, 7 mls of the dispersion was placed into a
15 14 ml glass vial with 3 ml of distilled water. The vial
16 was placed in warm, 35°C sonicating water bath with N₂
17 bubbling through it to evaporate the solvent. Once the
18 solvent has evaporated, the aqueous dispersion was then
19 suspended in a total of 10 mls of autoclaved water,
20 sonicated for one hour, and centrifuged for 5 minutes.
21 The supernatant was removed and brought to 20 mg
22 particle/ml solution with autoclaved water.

23

24 XI. Utility

25 The targeted wave absorbing magnetic core liposome may
26 be prepared to include ferrites useful as cancer
27 chemotherapeutic agents. In one method of synthesis, the
28 magnetic core liposomes are prepared to include PEG-PE and
29 PG on the liposome backbone to aid in targeting to
30 specific areas and to avoid RES uptake.

31 Magnetic liposome compositions are also useful for
32 radio-imaging or MRI imaging of solid tumor regions prior
33 to EM wave exposure and cell destruction. The magnetic
34 liposomes are prepared with encapsulated radio-opaque or
35 particle-emission metal oxides or ferrites which

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1 substantially prevents permeation through the magnetic
2 liposome bilayer.

3 In still another application, the magnetic liposome
4 composition is designed to enhance uptake of circulating
5 cells or other blood-borne particles, such as bacteria,
6 virus-infected blood cells and the like. Here the long-
7 life magnetic liposomes are prepared to include surface-
8 bound ligand molecules, as above, which bind specifically
9 and with high affinity to the selected blood-borne cells.
10 Once bound to the blood-borne particles, the magnetic
11 liposomes can be exposed to EM fields for specific cell or
12 virus destruction.

13 Other objects and advantages of this invention will
14 become apparent upon consideration of the following
15 working examples.

16

17

EXAMPLE 1

Preparation of Absorbing Ferrite by Thermal Processes

18 A mixture consisting of nickel oxide (NiO), zinc oxide
19 (ZnO), ferric oxide (Fe₂O₃) was mixed in a muller for 1
20 hour. The resulting powder was then screened through a 20
21 mesh screen. The powder was then treated in an oven at
22 350 degrees C. for 48 hours. The powder was then sintered
23 at 1260 degrees C. in contact with air for 24 hours, and
24 then cooled to room temperature over a period of 24 hours.
25 Powders of different compositions were manufactured by
26 varying the ratio of nickel oxide and zinc oxide in
27 accordance with the relationship NiO_xZnO-Fe₂O₄ where x is
28 varied between 0.3 and 1.0. Frequency range absorbances
29 are specified for some of the compositions in the
30 following table.
31

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Table 1

	<u>COMPOSITION</u>	<u>(X)</u>	<u>FREQUENCY RANGE (mHz)</u>	<u>%ABSORBANCE</u>
5	NiOZnOFe ₂ O ₄	.3	55 - 105	89
7	NiOZnOFe ₃ O ₄	.6	145 - 1040	66
9	NiOZnOFe ₂ O ₄	.9	530 - 2750	105

EXAMPLE 2Preparation of Ferrite by Hydroxide Gel Process

.148 moles of FeCl₃ was dissolved in 50ml distilled water then precipitated with 150ml of 1M NaOH. .037 moles of FeCl₂:4H₂O was dissolved in 50 ml distilled water then precipitated with 25ml of 1M NaOH. .0185Moles CaCl₂ was dissolved in 50ml distilled water and precipitated with 25ml of 1M NaOH. .0185 moles ZnCl₂ was dissolved in 50ml distilled water and precipitated with 25ml of 1M NaOH. All four precipitated solutions were added together in a large beaker and mixed vigorously for four min. in an industrial blender. The resulting gel was heated at 90 degrees C for 6 hours. O₂ was bubbled through the solution for the entire 6 hours.

EXAMPLE 3Preparation of Ferrite by Hydroxide Gel Process

.148 moles of FeCl₃ was dissolved in 50 ml distilled water then precipitated with 150ml of 1M NaOH. .037 moles of FeCl₂:4H₂O was dissolved in 50ml distilled water then precipitated with 25ml of 1M NaOH. .037 Moles MnCl₂ was dissolved in 50ml distilled water and precipitated with 25ml of 1M NaOH. All three precipitated solutions were added together in a large beaker and mixed vigorously in a blender for four min. The resulting gel was heated at 90 degrees C for 6 hours. O₂ was bubbled through the solution for the entire 6 hours.

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1 EXAMPLE 4

2 Preparation of Ferrite by Hydroxide Gel Process

3 .148 moles of FeCl_3 was dissolved in 50ml distilled
4 water then precipitated with 100ml of 0.1M LiOH. .037
5 moles of $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was dissolved in 50ml distilled water
6 then precipitated with 25ml 0.1M LiOH. Both precipitated
7 solutions were added together in a large beaker and mixed
8 vigorously for four min. The resulting gel was heated at
9 90 degrees C for 6 hours. O_2 was bubbled through the
10 solution for the entire 6 hours.

11

12 EXAMPLE 5

13 Preparation of Ferrites from Hydroxide Gels

14 A reactor provided with a heat exchange coil and a
15 radial stirrer, was fed with 3600 ml of ferrous sulphate
16 solution having a concentration of 40 g/liter.
17 Subsequently, 290 ml of ammonia solution (200 g/liter of
18 NH_3) were added thereto, while stirring at 100 rpm. Such
19 stirring was carried on throughout the first step. Air
20 was blown into the reactor at a flow rate of 100 cc/hr.
21 and the temperature was kept at about 30 Deg. C by cooling
22 the heat exchange coil with water. The first step of the
23 reaction was concluded when the pH value decreased to 3.5
24 and the platinum electrode, with respect to the calomel
25 electrode, indicated +110mV. This occurred about 7 hours
26 after the beginning of the flowing in of air.

27 The analysis of the slurry was as follows:

28 $\text{Fe}^{++} = 11.1\text{g/liter}$; $\text{Fe} = 37.1\text{g/liter}$.

29 160 ml of a ferrous sulphate solution (63.5 g/liter of
30 Fe^{++}) were admixed with the slurry. After this
31 adjustment, the analysis of the slurry was as follows:
32 $\text{Fe}^{++} = 13.1\text{g/liter}$; $\text{Fe} = 38.5\text{g/liter}$, the FeII/FeIII
33 ratio being 0.52.

34 The reactor was fed with 155 ml of an ammonia solution
35 (195 g/liter of NH_3) with stirring at 110 rpm. This
36 stirring was continual throughout the second step. The

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1 temperature was brought to 90 degrees C. by conveying
2 steam into the heat exchange coil, and the temperature was
3 kept constant by means of a thermostat. During the
4 reaction the pH value decreased from 8 to about 6.5. The
5 second step of the reaction was terminated when the redox
6 potential rose from -700 to about -450 mV. This occurred
7 about 3 hours from the beginning of the heating. At the
8 end, the ferrous iron present as $\text{Fe}(\text{OH})_2$ was 0.34 g//liter
9 of FeII. The slurry was acidified to a pH value = 4 to
10 remove ferrous hydroxide. The magnetic particles, once
11 filtered, washed and dried, exhibited the following
12 characteristics:

13	Morphology	Cubic
14	Average Diameter	
15	d10	0.182
16	Numerical variancy	
17	Coefficient	22.0%
18	Mg content	0.04%
19	S content	0.61%
20	Specific surface	6.52 m ² /g
21	magnetization	5680 G/domain

22

23 EXAMPLE 6

24 Preparation of Oleic Acid Coated Magnetic Particles

25 Wave absorbing magnetic particles, coated with oleic acid
26 were prepared using the ferrites prepared in Examples 1-5.

27 The ferrite powder is dispersed in a beaker with
28 approximately 1500 cc distilled water, adjusted to a
29 concentration of approximately 10 wt % and stirred with a
30 paddle stirrer for about 5 minutes. The beaker containing
31 the ferrite slurry is then placed onto a permanent magnet,
32 separating the wave absorbing magnetic particle from the
33 aqueous salt waste solution. After resting the slurry on
34 the magnet for 5 minutes, the aqueous salt solution is
35 decanted. The precipitate is then resuspended by
36 agitation in an additional 1500 cc of fresh distilled

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1 water. After the final water wash is decanted, the
2 particles are suspended in acetone and the above washing
3 procedure is repeated 5 times. The particles are then
4 washed with hexane a total of five times each in the above
5 manner.

6 Oleic acid is added to the magnetic particle/hexane
7 slurry in a ratio of 2:1 oleic acid:dry particle. The
8 mixture is adjusted to 15% total solids with hexane and
9 milled overnight on a mechanical jar roller in a glass jar
10 half filled with 3mm stainless steel balls.

11 The samples were labeled 1-5 to correspond to the
12 ferrites prepared in Examples 1-5.

13

14

EXAMPLE 7

15

Preparation of Phenyl Lipid

16

A. Synthesis of a m-isophthalic acid based phenyl
17 lipid.

18

The starting material for this synthesis is
19 5-Aminoisophthalic acid. The 5-aminoisophthalic acid is
20 not soluble in dioxane alone. It is soluble in a mixture
21 of dioxane and triethylene glycol. 5-aminoisophthalic
22 acid (145 mg) was dissolved in 5 ml. of dioxane and 2 ml.
23 of triethylene glycol, and the pH was adjusted to 10 with
24 NaOH. Methoxypolyoxyethylene imidazoly carbonyl, average
25 mol. wt. 5,000 from Sigma (2.0g) was dissolved in 2ml of
26 H₂O, 1.0ml of 1N Na₂CO₃, and 2.0 ml of triethylene glycol.
27 This solution was added to the 5-aminoisophthalic acid
28 solution and stirred for 36 hours at room temperature.
29 The reaction mixture was then dialyzed overnight against 2
30 liters of H₂O. The dialyzed reaction mixture was mixed
31 with 100ml of pyridine and the liquids removed via rotary
32 evaporation. The resulting yellow oil was placed in the
33 refrigerator. After several days a white precipitate
34 formed. The precipitate contains both coupled and
35 uncoupled PEG.

36

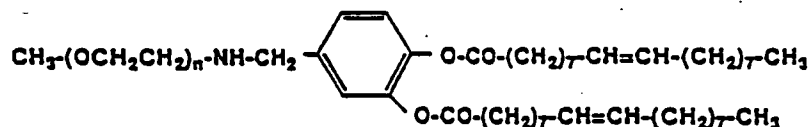
Oleyl alcohol can be coupled to the above isophthalic

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1 acid derivative using thionyl chloride. The thionyl
 2 chloride can be used to activate the oleyl alcohol for
 3 ester formation with the carboxyl groups of the
 4 isophthalate. See. Fig. 2.

5 B. Synthesis of ortho phenyl lipids

6 The ortho analog of the phenyl lipids can be
 7 synthesized starting with either 3,4 dihydroxybenzaldehyde
 8 or 3,4 dihydroxybenzoic acid. The aldehyde group can be
 9 coupled to an amino group by forming the Schiff's base and
 10 then reducing it with NaBH_4 . Oleic acid could then be
 11 coupled to the hydroxyl groups using thionyl chloride to
 12 provide:



13
 14
 15
 16
 17
 18 3,4 dihydroxybenzoic acid could be coupled through
 19 its carboxyl group to amino-terminated PEG using
 20 dicyclohexyl carbodiimide. Oleic acid could then be
 21 coupled as above.

22 Since both amino and carboxyl PEG derivatives as well
 23 as both oleic acid and oleylamine are available, the PEG
 24 and oleic acid groups can be easily interchanged in the
 25 above compounds.

26 EXAMPLE 8

27 Preparation of Magnetic Liposomes

28 Using Phosphatidyl Choline

29
 30 10 grams of each of the oleic acid coated ferrite as
 31 prepared in Example 6 were dispersed in 100 cc hexane.
 32 The phospholipid was absorbed onto the particle by
 33 dissolving phosphatidyl choline (Sigma, P-3644, L-2
 34 lecithin, 45% PC) into hexane with heating to create a 15%
 35 solution. The PC/hexane solution was combined with the
 36 magnetic particles/hexane solution at a ratio of pure

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1 phosphatidyl choline:oleic acid equal to 1:2 weight
2 percent.

3 The solution was mixed in a glass jar (without media)
4 on a jar roller for two hours. 50 cc of distilled water
5 were added to the jar and mixing was continued for an
6 additional 1 hour. The jar and its contents were then
7 transferred to an ultrasonic bath and treated by
8 ultrasound for an additional 30-60 minutes.

9 The slurry was transferred to a 200 cc beaker and
10 heated on a hot plate to 100 deg C for 10 min. From .05
11 to 50 grams of triton x-114 (Union Carbide) was added to
12 disperse the lipidized ferrite in water. A ratio of
13 triton X114:lipid particle equal to 1:6 weight percent was
14 the optimum level for the dispersion. The dispersion was
15 mixed on a laboratory vortex mixer for 2 minutes and
16 placed in an ultrasonic bath (Branson 1200, VWR) for two
17 hours. The final dispersion was adjusted to 0.2% TS
18 (2mg/ml). Particles were measured on a Nycomp laser
19 particle size analyzer and were found to be approximately
20 200 nm in diameter.

21

22

EXAMPLE 9

Preparation of Magnetic Liposomes using Phenyl Lipid

24 Samples were prepared using particles from Examples
25 1-5 exactly as described in Example 8 except that phenyl
26 lipids prepared in Example #7 was used in place of PC.

27 Samples were labeled for later i.d. 6-10 to correspond
28 with the particles as prepared in Examples 1-5. Samples
29 were measured for particle size on a nycomp particle
30 analyzer and found to be approximately 200 nm in diameter.

31

EXAMPLE 10

Preparation of MDCK Cell Cultures

33 Upon the arrival, ampules of CCL34, MDCK cells (NBL-2
34 canine kidney) from ATCC, are quickly thawed. Using a
35 sterile Pasteur pipette the contents of the ampule are
36 transferred to a flask containing at least 10 volumes of

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1 culture medium (Eagles MEM) previously adjusted to pH 7.4.
2 The cells are incubated for 24 hours, the media is
3 withdrawn, discarded and replaced. Cells are incubated at
4 36.5 degrees C. in a CO₂ incubator for approximately 7
5 days. Another medium change may be necessary if indicated
6 by a drop in pH or high cell concentration.

7 Cells are transferred during log phase, once
8 confluence has been reached. The procedure is as follows:
9 The media is withdrawn and discarded. A PBSA (5ml/25cm²)
10 prewash is added to the flask opposite the cell monolayer.
11 To avoid disruption the cells are rinsed and the solution
12 discarded. Next, 3 ml/25 cm² trypsin is added to the
13 flask (opposite of cells). The flask is turned to expose
14 the cells to the trypsin for 15-30 seconds, then the
15 trypsin is discarded making sure the monolayer is not
16 detached. The cells are incubated until the monolayer
17 will slide down the flask surface when tipped.
18 (Approximately 5-15 min.) MEM medium is used to disperse
19 the cells by repeated pipetting. Cells are diluted to
20 10-100 cells/ml and seeded in transwells as follows:
21 Costar 6 well transwell-COL(3418) with pore size of 3.0
22 micron and well and 1.5ml of culture (media and cells) are
23 added to the inside of the transwell. The wells are
24 covered and incubated until the monolayer is established
25 on the membrane. The cell cultures thus prepared were
26 used for all further experiments.

27 EXAMPLE 11

28 Ferrites were prepared as described in Examples 1-5,
29 coated with oleic acid as in Example #6 and treated with a
30 second layer of phenyl lipid as described in Example #7.

31 A culture of MDCK cells were prepared as described in
32 Example #10. The lipid coated ferrites and uncoated
33 (bare) ferrite controls were put in contact with the MDCK
34 cells grown above a colony of rat brain cancer cells

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1 (neuroblastoma) as detailed in the figure below.

2

3

4

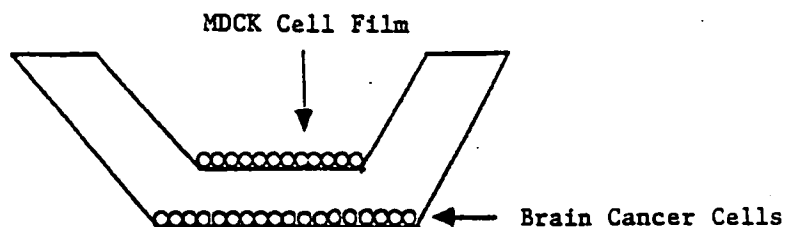
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6

7

8

9



10 The sample was allowed to incubate at room temperature
11 for a period of 1 hour, then exposed to a frequency of
12 20000 mHz for 3 minutes. None of the bare ferrite were
13 permeable to the endothelial cell (MDCK) membrane and had
14 no effect on the cancer cell colony.

15 Ferrites as prepared in Example 1, 2, 3 and 4 rapidly
16 heated upon exposure to the EM wave and all the brain
17 cells in the culture were killed.

18 Ferrites as prepared in Sample #5 were able to cross
19 the endothelial cell barrier, however, because they are
20 all iron, do not absorb EM waves and had no effect on the
21 neuroblastoma cells.

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CLAIMS

1

2

3 1. A coated magnetic or superparamagnetic responsive
4 particle comprising:

5 a. a magnetic core particle comprising a
6 magnetically-responsive metal, metal alloy or metal oxide;
7 and

8 b. an organo-metallic polymer coating covalently
9 bonded to or absorbed onto said particle wherein the
10 bonding or adsorption does not depend upon the presence of
11 hydroxy functionality on the surface of said particle, and
12 wherein the organo-metallic polymer coating preferably has
13 functional groups selected from the group consisting of
14 amino, carboxyl, hydroxyl, sulfate, phosphate, vinyl,
15 nitrate, aldehyde, epoxy, succinamine, anhydride, cyanate,
16 and thiol groups, and is capable of binding at least one
17 type of bioaffinity adsorbent, preferably selected from
18 the group consisting of antibodies, antigens, enzymes and
19 specific binding proteins.

20 2. A coated magnetically responsive particle of
21 claim 1, wherein the magnetic core particle comprises a
22 metal, metal alloy or metal oxide selected from the group
23 consisting of iron, magnetite, iron magnesium oxide, iron
24 manganese oxide, iron cobalt oxide, iron nickel oxide,
25 iron zinc oxide and iron copper oxide, preferably
26 containing a particle size of from about 0.003 to about
27 1.5 microns in diameter, wherein the organo-metallic
28 polymer is preferably formed from monomers which are
29 coordinate complexes of organic ligands and a metal
30 selected from the group consisting of: titanium,
31 zirconium, hafnium, vanadium, tantalum, niobium, tin,
32 antimony, zinc, cadmium, manganese, tellurium, rhenium,
33 aluminum, gallium, germanium and iridium, or wherein the
34 organo-metallic polymer is preferably an organo-titanium
35 polymer selected from the group consisting of: titanium-
36 tetra-isopropoxide, amino-hexyl-titanium-triisopropoxide,

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1 amino-propyl-titanium-triisopropoxide and carboxyl-hexyl-
2 titanium triisopropoxide.

3 3. A method of measuring analytes in a sample
4 comprising the steps of:

5 a. contacting a sample containing an unknown
6 concentration of the analyte with a known amount of a
7 labeled analyte in the presence of magnetic particles
8 comprising:

9 (i) a magnetic core particle comprising a
10 magnetically responsive metal, metal alloy or metal oxide;
11 and

12 (ii) an organo-metallic polymer coating
13 covalently bonded to or adsorbed onto said particle
14 wherein the bonding or adsorption does not depend upon the
15 presence of hydroxy functionality on the surface
16 particles, and wherein said organo-metallic coating has a
17 bioaffinity adsorbent covalently coupled thereto, said
18 bioaffinity adsorbent is capable of binding to or
19 interacting with both the unlabeled and the labeled
20 analyte;

21 b. maintaining the mixture in step (a) under
22 conditions sufficient for said binding or interaction to
23 occur;

24 c. magnetically separating the magnetic
25 particles; and

26 d. measuring the amount of label associated with
27 the magnetic particles and determining the concentration
28 of analyte in solution.

29 4. The method of claim 3 wherein the analyte is
30 preferably selected from the group consisting of:
31 antibodies, antigens, haptens, enzymes, apoenzymes,
32 enzymatic substrates, enzymatic inhibitors, cofactors,
33 nucleic acids, binding proteins, carrier proteins,
34 compounds bound by binding proteins, compounds bound by
35 carrier proteins, lectins, monosaccharides,
36 polysaccharides, hormones, receptors, repressors and

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1 inducers; wherein the magnetic core particle preferably
2 comprises a metal, metal alloy or metal oxide selected
3 from the group consisting of: iron, magnetite, iron
4 magnesium oxide, iron manganese oxide, iron cobalt oxide,
5 iron nickel oxide, iron zinc oxide and iron copper oxide,
6 and preferably has a particle size of from about 0.003 to
7 about 1.5 microns in diameter; wherein the organo-metallic
8 polymer coating is preferably formed from monomers which
9 are coordinate complexes of organic ligands and a metal
10 selected from the group consisting of: titanium,
11 zirconium, hafnium, vanadium, tantalum, niobium, tin,
12 antimony, zinc, cadmium, manganese, tellurium, rhenium,
13 aluminum, gallium, germanium and iridium; wherein the
14 organo-metallic polymer is more preferably an organo-
15 titanium polymer selected from the group consisting of:
16 titanium-tetra-isopropoxide, amino-hexyl-titanium
17 triisopropoxide, amino-propyl-titanium isopropoxide and
18 carboxyl-hexyl-titanium triisopropoxide; wherein the
19 magnetically responsive particle is preferably
20 superparamagnetic; wherein the bioaffinity adsorbent is
21 preferably selected from the group consisting of:
22 antibodies, antigens, haptens, enzymes, apoenzymes,
23 enzymatic substrates, enzymatic inhibitors, cofactors,
24 nucleic acids, binding proteins, carrier proteins,
25 compounds bound by binding proteins, compounds bound by
26 carrier proteins, lectins, monosaccharides,
27 polysaccharides, hormones, receptors, repressors and
28 inducers; and wherein the labeled analyte is preferably
29 marked with a label selected from the group consisting of:
30 radioisotopes, fluorescent compounds, enzymes and
31 chemiluminescent compounds.

32 5. A method for preparing inorganic oxides of
33 substantially uniform particle size distribution
34 comprising contacting aqueous solutions of an inorganic
35 salt and an inorganic base across a porous membrane
36 wherein the membrane contains a plurality of pores which

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1 allows for precipitation of a substantially mono-dispersed
2 size inorganic oxide particles on one side of the membrane
3 and precipitation of a salt of the corresponding base on a
4 second side of the membrane.

5 6. The method of claim 5 wherein the particle size
6 diameter is preferably 20, 50, 80 or 100A; and wherein the
7 particle size distribution is preferably +/- 10%; wherein
8 the inorganic salt is preferably of the formula MY,
9 wherein M is selected from the group consisting of Fe, Co,
10 Ni, Zn, Mn, Mg, Ca, Ba, Sr, Cd, Hg, Al, B, Sc, Ga, V In,
11 and mixtures thereof; and wherein the inorganic salt is of
12 the formula MY, and Y is preferably selected from the
13 group consisting of Cl, Br, I, SO₄, NO₃, PO₄ and mixtures
14 thereof; and wherein the inorganic base is preferably
15 selected from the group consisting of NH₄OH, KOH, LiOH,
16 NaOH, CsOH, RbOH and mixtures thereof; and wherein the
17 substantially mono-dispersed precipitated inorganic oxide
18 particle size is preferably from about 5-1000A in
19 diameter; and wherein the substantially mono-dispersed
20 precipitated inorganic oxide particle is of the formula
21 M₃O₄ wherein M is preferably selected from the group
22 consisting of Fe, Co, Ni, Zn, Mn, Mg, Ca, Ba, Sr, Cd, Hg,
23 Al, B, Sc, Ga, V, In and mixtures thereof; and wherein the
24 substantially mono-dispersed precipitated inorganic oxide
25 particle is preferably Fe₃O₄; and wherein the size of the
26 precipitated inorganic oxide particle is preferably
27 increased by selecting an inorganic base with a relatively
28 rapid dissociation constant; and wherein the size of the
29 precipitated inorganic oxide particle is preferably
30 reduced by selection of an inorganic base with a
31 relatively slow dissociation constant; and wherein the
32 size of the precipitated inorganic oxide particles is
33 further controlled by varying the pore size of the
34 membrane, the temperature of the inorganic salt and
35 inorganic base solutions, and the concentration of the
36 aqueous inorganic salt solution; and wherein the

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1 concentration of the aqueous inorganic salt solution is
2 preferably about 1-3%wt; and wherein the size of the
3 precipitated particles is controlled by adjusting the
4 concentration of the aqueous inorganic base; and wherein
5 the concentration of the aqueous solution of inorganic
6 base is preferably about 2-4%wt; and wherein the aqueous
7 inorganic salt solution and the aqueous inorganic base are
8 preferably allowed to remain in contact across said
9 membrane for a period of about 40-80 hours; and wherein
10 said membrane is preferably selected from material
11 consisting of cellulose polymer, a fluropolymer, a
12 chlorinated olefin polymer, and a polyamide; and wherein
13 the pore size of the membrane as measured by the molecular
14 weight cut-off is preferably adjusted between 1000 and
15 500,000.

16 7. A controllably degradable aggregate cluster
17 comprising a cluster of inorganic oxides of substantially
18 mono-dispersed particle size which are coated with a
19 functionalized organic moiety wherein the cluster is
20 bonded together by chemical, complex, or ionic coupling
21 between the functional groups of said organic moiety.

22 8. The controllably degradable aggregate cluster of
23 claim 7 wherein the functionalized organic moiety is
24 preferably an organo-metallic polymer; and wherein the
25 organo-metallic polymer coatings are formed from organo-
26 metallic monomers selected from the group consisting of:
27 amino-hexyl-titanium triisopropoxide; amino-propyl-
28 titanium triisopropoxide and carboxy-hexyl-titanium
29 triisopropoxide; and wherein the aggregate cluster is
30 preferably superparamagnetic and the individual particles
31 are non-magnetic.

32 9. A controllable degradable aggregate bead cluster
33 which comprises:

34 a cluster of inorganic oxide particles of
35 substantially mono-dispersed particle size associated with
36 a macromolecular species, characterized in that said

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1 particles are encapsulated by the macromolecular species
2 forming a bead, the macromolecular species containing
3 organic functionality to link the beads together forming
4 controllably degradable chemical, complex, or ionic bonds.

5 10. The controllably degradable aggregate bead
6 cluster of claim 9 wherein the macromolecular species is
7 selected from the group consisting of polystyrene,
8 poly(vinyl chloride) and polyurethane; and wherein the
9 bead is preferably formed by surrounding the particles
10 with a difunctional organic monomer, one functionality of
11 the monomer adsorbed onto or covalently bound to the
12 particles, one functionality covalently bonded as between
13 monomers forming macromolecular encapsulation; and wherein
14 the aggregate bead cluster is preferably
15 superparamagnetic, and the individual beads are non-
16 magnetic.

17 11. A method for determining the concentration of a
18 ligate in solution which comprises:

19 a. providing a substantially mono-dispersed
20 inorganic oxide particle of claim 5 wherein said particles
21 are non-magnetic;

22 b. coating said particles with an organo-
23 metallic polymer coating which is adsorbed onto or
24 covalently bound to the particle and which is
25 functionalized to covalently bind to a ligand moiety
26 having specific affinity for the ligate to be measured;

27 c. covalently binding said ligand moiety to the
28 particle;

29 d. reacting the product in step (c) with a
30 solution containing the ligate to be measured to form a
31 ligand/ligate magnetic complex;

32 e. relating the magnetic response of the product
33 in step (d) to the concentration of the ligate causing the
34 complexation.

35 12. The method of claim 30 wherein the ligand is an
36 antibody and the antibody is preferably selected from the

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1 group consisting of anti-thyroxine, anti-triiodothyronine,
2 anti-thyroid stimulating hormone, anti-thyroid binding
3 globulin, anti-thyroglobulin, anti-digoxin, anti-cortisol,
4 anti-insulin, anti-theophylline, anti-vitamin B-12, anti-
5 folate, anti-ferritin, anti-human chorionic gonadotropin,
6 anti-follicle stimulating hormone, anti-progesterone,
7 anti-testosterone, anti-estriol, anti-estradiol, anti-
8 prolactin, anti-human placental lactogen, anti-gastrin and
9 anti-human growth hormone antibodies; and wherein the
10 ligate is preferably selected from the group consisting of
11 hormones, peptides, pharmacological agents, vitamins,
12 cofactors, hematological substances, virus antigens,
13 nucleic acids and nucleotides; and wherein the ligate is
14 more preferably selected from the group consisting of
15 thyroxine, theophylline, vitamin B-12, triiodothyronine,
16 and thyroid stimulating hormone, and the ligand is
17 selected from the group consisting of anti-theophylline
18 anti-body, vitamin B-12 binding protein, and anti-thyroid
19 stimulating hormone anti-body.

20 13. A method for determining the concentration of a
21 metal in solution which comprises:

22 a. providing a substantially mono-dispersed
23 inorganic oxide particle of claim 1 wherein said particles
24 are non-magnetic;

25 b. coating said particles with an organo-
26 metallic polymer coating which is adsorbed onto or
27 covalently bound to the particle and which is
28 functionalized to covalently bind to an organic moiety
29 having specific affinity for the metal to be measured;

30 c. covalently binding said organic moiety to the
31 particle;

32 d. reacting the product in step (c) with a
33 solution containing the metal to be measured to form a
34 magnetic complex;

35 e. relating the magnetic response of the product
36 in step (d) to the concentration of the metal causing the

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1 complexation.

2 14. The method of claim 13 wherein the organic moiety
3 having specific affinity for a metal to be measured is
4 preferably 2,3-dihydroxy-5-benzoic acid; and wherein the
5 metal to be measured is preferably selected from the group
6 consisting of Tu and Mo; and wherein the organic moiety
7 having specific affinity for the metal to be measured is
8 preferably 2,3-dithio-5-benzoic acid and the metal to be
9 measured is Mo.

10 15. A liposome composition comprising a substantially
11 uniform size inorganic core coated with an amphipathic
12 organic compound and further coated with a second
13 amphipathic vesicle forming lipid.

14 16. The liposome composition of claim 15 wherein the
15 inorganic core is preferably selected from the group
16 consisting of Fe_3O_4 , Fe_2O_3 , Al_2O_3 , TiO_2 , ZnO , FeO and Fe ;
17 and wherein the inorganic core is preferably a
18 substantially uniform sub 100 nm diameter inorganic oxide;
19 and wherein the amphipathic organic compound is preferably
20 a fatty acid selected from the group consisting of oleic,
21 linoleic, linolenic, palmitic, myristic and arachidonic
22 acid; and wherein the vesicle forming lipid is preferably
23 selected from the group consisting of phospholipids,
24 sterol lipids and glycolipids; and wherein the
25 phospholipid is preferably selected from the group
26 consisting of phosphatidylcholine, phosphatidic acid and
27 phosphatidylinositol.

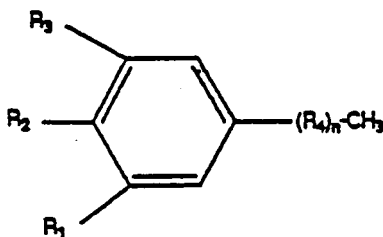
28 17. A liposome composition for use in delivering a
29 compound via the bloodstream comprising a substantially
30 uniform size inorganic core coated with an amphipathic
31 organic compound and further coated with 1-20 mole percent
32 of an amphipathic vesicle-forming lipid derivatized with a
33 hydrophilic polymer, and containing the compound in
34 liposome-entrapped form.

35 18. The composition of claim 17 wherein the
36 hydrophilic polymer is preferably selected from the group

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1 consisting of poly(ethylene oxide), poly(propylene oxide)
2 and poly(vinyl alcohol); and wherein the liposomes
3 preferably have a selected average size in the size range
4 between about 5 and 5000 nanometers; and wherein the
5 hydrophilic polymer preferably has a molecular weight
6 between about 1,000 to 5,000 daltons; and wherein the
7 vesicle forming lipid is preferably selected from the
8 group consisting of phospholipids, sterol lipids, and
9 glycolipids; and wherein the phospholipid is preferably
10 derivatized with poly(ethylene oxide); and wherein the
11 phospholipid is preferably phosphatidylethanolamine and
12 the poly(ethylene oxide) is coupled to the
13 phosphatidylethanolamine through a lipid amine group.

14 19. A synthetic vesicle forming phenyl lipid compound
15 having the structural formula:

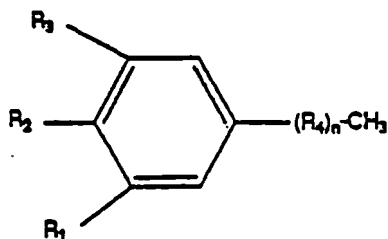


23 wherein two of R₁, R₂ and R₃ represent saturated or
24 unsaturated straight-chain or branched chain alkyl or acyl
25 groups, the other being hydrogen, and R₄ is an alkylene
26 oxide or vinyl alcohol repeat unit and n varies from 0 to
27 about 200.

28 20. A liposome composition for use in delivering a
29 compound via the bloodstream containing the compound in
30 liposome entrapped form comprising a substantially uniform
31 size inorganic core coated with an amphipathic compound
32 and further coated with 1-20 mole percent of an
33 amphipathic vesicle-forming phenyl lipid having the

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1 formula:



8 wherein two of R₁, R₂ and R₃ represent saturated or
9 unsaturated straight-chain or branched chain alkyl or acyl
10 groups, the other being hydrogen, and R₄ is an alkylene
11 oxide or vinyl alcohol repeat unit and n varies from 0 to
12 about 200.

13 21. The composition of claim 20 wherein the alkylene
14 oxide repeat unit is preferably selected from the group
15 consisting of ethylene oxide and propylene oxide; and
16 wherein the branched chain alkyl or acyl groups are
17 organic radicals preferably derived from the group
18 consisting of oleic acid, stearic acid, linoleic acid,
19 linolenic acid, palmitic acid, myristic acid, and
20 arachidonic acid.

21 22. A method for preparing a substantially uniform
22 size inorganic core liposome composition comprising the
23 steps of preparing a substantially uniform size organic
24 oxide particles, coating said particle with an amphipathic
25 organic compound wherein the organic compound is adsorbed
26 or coordinated onto the surface of the inorganic oxide,
27 and associating said coated particle with an amphipathic
28 vesicle forming lipid.

29 23. The method of claim 22 wherein the substantially
30 uniform size inorganic oxide particle is preferably
31 prepared by contacting aqueous solutions of an organic
32 salt and an inorganic base across a porous membrane
33 wherein the membrane contains a plurality of pores which
34 allows for precipitation of a substantially uniform size
35 inorganic oxide particle on one side of the membrane and
36 precipitation of a salt of the corresponding base on a

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1 second side of the membrane; and wherein the substantially
2 monodispersed precipitated inorganic particle size is
3 preferably from about 5-1000A in diameter; and wherein the
4 substantially monodispersed precipitated inorganic oxide
5 particle is preferably Fe_3O_4 .

6 24. A composition comprising a wave absorbing
7 magnetic core particle coated with an amphipathic organic
8 compound and further coated with a second amphipathic
9 vesicle forming lipid.

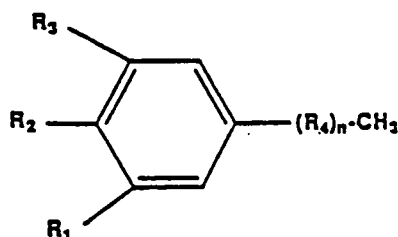
10 25. The composition of claim 24 wherein the wave
11 absorbing magnetic core particle is a ferrite material of
12 the formula $\text{M}_2(+3)\text{M}(+2)\text{O}_4$, wherein the $\text{M}(+3)$ is preferably
13 selected from the group consisting of Al, Cr and Fe, and
14 $\text{M}(+2)$ is preferably selected from the group consisting of
15 Fe, Ni, Co, Zn, Zr, Ca, Ba, Mg, Ga, Gd, Mn and Cd; and
16 wherein the ferrite material is preferably mixed with LiO ,
17 NaO , KO , Fe_2O_3 or Fe_3O_4 ; and wherein the wave absorbing
18 magnetic core particle is preferably a substantially
19 uniform sub 100 nm diameter ferrite particle; and wherein
20 the amphipathic organic compound is preferably a fatty
21 acid selected from the group consisting of oleic,
22 linoleic, linolenic, palmitic, myristic and arachidonic
23 acid; and wherein the vesicle forming lipid is preferably
24 selected from the group consisting of phospholipids,
25 sterol lipids and glycolipids; and wherein the
26 phospholipid is preferably selected from the group
27 consisting of phosphatidylcholine, phosphatidic acid,
28 phosphatidylinositol, and phosphatidyl ethanolamine.

29 26. A liposome composition for use in delivering a
30 compound via the bloodstream comprising a wave absorbing
31 magnetic core coated with an amphipathic organic compound
32 and further coated with 1-20 mole percent of an
33 amphipathic vesicle-forming lipid derivatized with a
34 hydrophilic polymer, and containing the compound in
35 liposome-entrapped form.

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1 27. The composition of claim 26 wherein the
 2 hydrophilic polymer is preferably selected from the group
 3 consisting of poly(ethylene oxide), poly(propylene oxide)
 4 and poly(vinyl alcohol); and wherein the liposomes
 5 preferably have a selected average size in the size range
 6 between about 5 and 5000 nanometers; and wherein the
 7 hydrophilic polymer preferably has a molecular weight
 8 between about 1,000 to 5,000 daltons; and wherein the
 9 vesicle forming lipid is preferably selected from the
 10 group consisting of phospholipids, sterol lipids, and
 11 glycolipids; and wherein the phospholipid is preferably
 12 derivatized with poly(ethylene oxide); and wherein the
 13 phospholipid is preferably phosphatidylethanolamine and
 14 the poly(ethylene oxide) is coupled to the
 15 phosphatidylethanolamine through a lipid amine group.

16 28. A liposome composition for use in delivering a
 17 compound via the bloodstream containing the compound in
 18 liposome entrapped form comprising a wave absorbing
 19 magnetic core coated with an amphipathic compound and
 20 further coated with 1-20 mole percent of an amphipathic
 21 vesicle-forming phenyl lipid having the formula:



28 wherein two of R_1 , R_2 and R_3 represent saturated or
 29 unsaturated straight-chain or branched chain alkyl or acyl
 30 groups, the other being hydrogen, and R_4 is an alkylene
 31 oxide or vinyl alcohol repeat unit and n varies from 0 to
 32 about 200.

33 29. The composition of claim 28 wherein the alkylene
 34 oxide repeat unit is preferably selected from the group
 35 consisting of ethylene oxide and propylene oxide; and
 36 wherein the branched chain alkyl or acyl groups are

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1 organic radicals preferably derived from the group
2 consisting of oleic acid, stearic acid, linoleic acid,
3 linolenic acid, palmitic acid, myristic acid, and
4 arachidonic acid.

5 30. A process for the preparation of substantially
6 uniform size oxides of the formula $M_2(+3)M(+2)O_4$
7 comprising:

8 supplying separate aqueous metal solutions of
9 $M(+3)$ and $M(+2)$;

10 adding alkali to said aqueous solutions and
11 precipitating the corresponding metal hydroxide; and
12 mixing the metal hydroxide precipitates in
13 solution together and heating to dehydrate, wherein the
14 dehydration temperature, time of dehydration, and
15 concentration of oxygen or air passed through the solution
16 are adjusted to control the particle size of the oxide
17 particle produced.

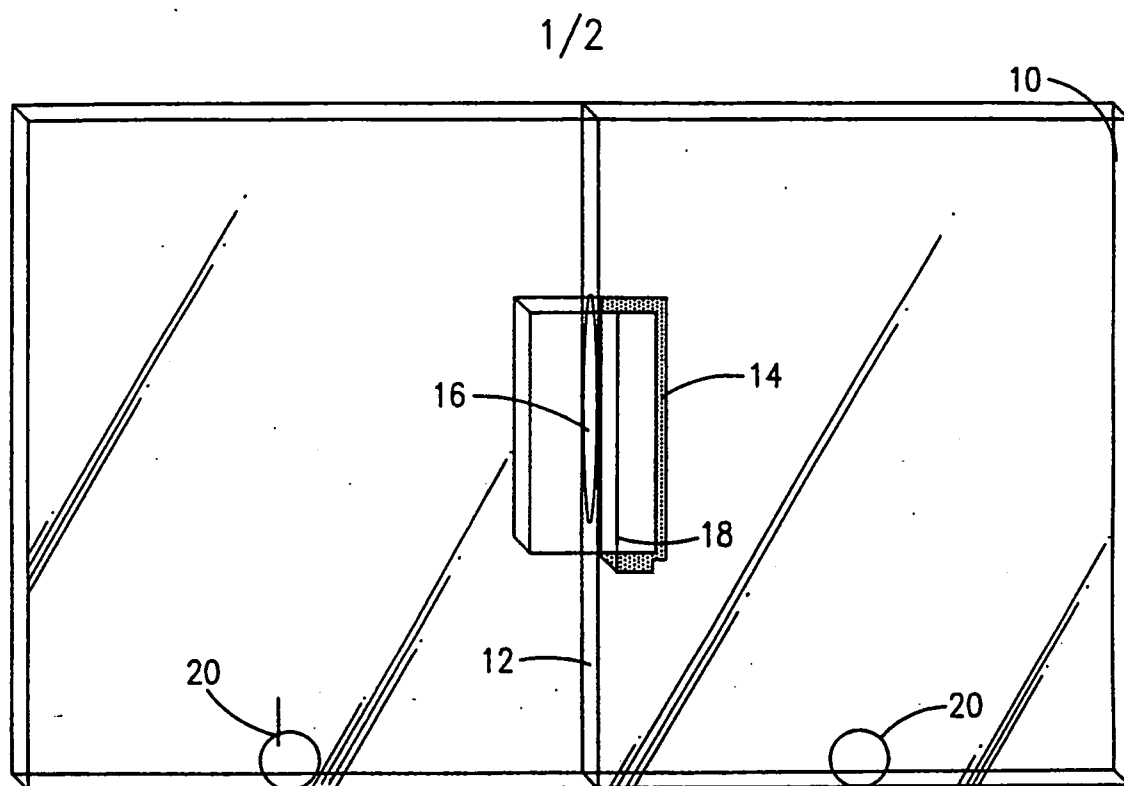
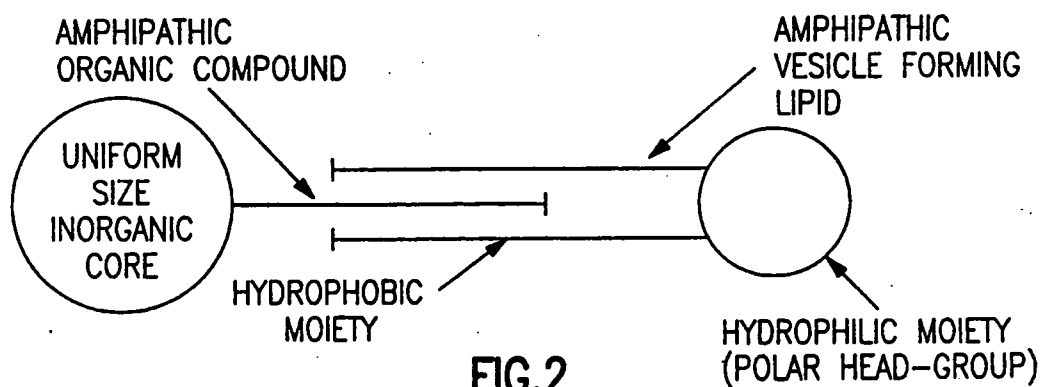
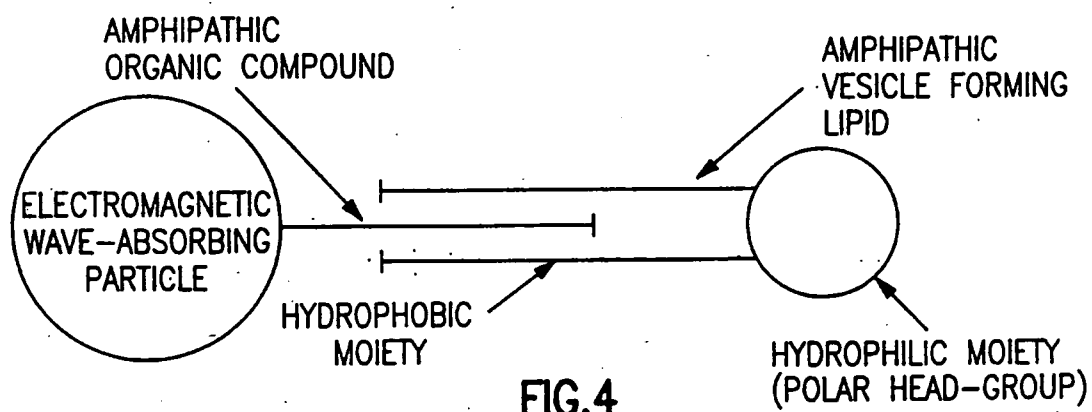
18 31. The process of claim 19 wherein $M(+3)$ is
19 preferably selected from the group consisting of Al, Cr
20 and Fe, and $M(+2)$ is preferably selected from the group
21 consisting of Fe, Ni, Co, Zn, Zr, Ca, Ba, Mg, Ga, Gd, Mn
22 and Cd; and wherein the dehydration temperature is
23 preferably 100°C and the dehydration temperature is 6
24 hours.

25 32. A method for preparing a wave absorbing magnetic
26 core liposome composition comprising the steps of
27 supplying wave absorbing magnetic core particles, coating
28 said particles with an amphipathic organic compound,
29 preferably an organometallic compound, wherein the organic
30 compound is adsorbed or coordinated onto the surface of
31 the said particle, and associating said coated particle
32 with an amphipathic vesicle forming lipid.

33 33. The process for the treatment of cancer cells or
34 infectious disease organisms by application of external
35 electromagnetic energy capable of the generation of heat
36 in intracellular particles to induce selective thermal
37 death of cancer cells comprising:

1 placing within the patient wave absorbing
2 magnetic core particles coated with an amphipathic organic
3 compound and further coated with a second amphipathic
4 vesicle forming lipid,
5 absorbing said coated wave absorbing magnetic
6 core particle intracellularly into the cancer cells,
7 subjecting the patient to an alternating
8 electromagnetic field to inductively heat the magnetic
9 core particle and thereby the cancer cells, and
10 continuing the inductive heating of said magnetic
11 core particle to attain an increase in intracellular
12 temperature to selectively kill either the cancer cells or
13 said organism.

14 34. The process of claim 33 wherein the magnetic
15 particles are ferrites, whose oxide component is of the
16 formula $M_2(+3)MO_4$, wherein $M(+3)$ is preferably selected
17 from the group consisting of Al, Cr and Fe, and M is
18 preferably selected from the group consisting of Fe, Ni,
19 Co, Zn, Zr, Ca, Ba, Mg, Ga, Gd, Mn and Cd; and wherein the
20 wave absorbing magnetic core is preferably a substantially
21 uniform size wave absorbing magnetic core particle
22 preferably in the range of from about 1 to 50,000 nm in
23 diameter.

**FIG. 1****FIG. 2****FIG. 4**

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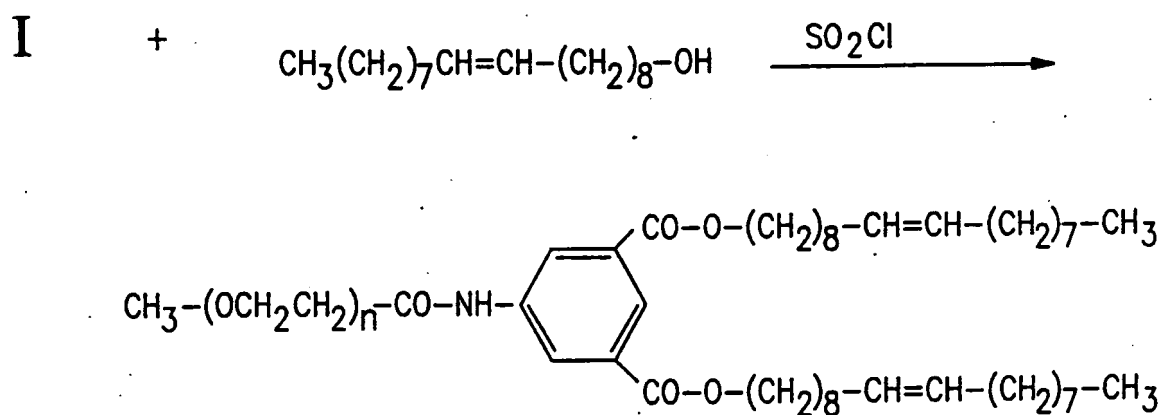
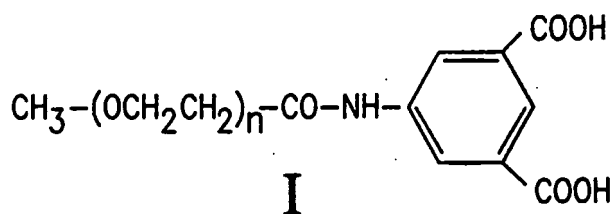
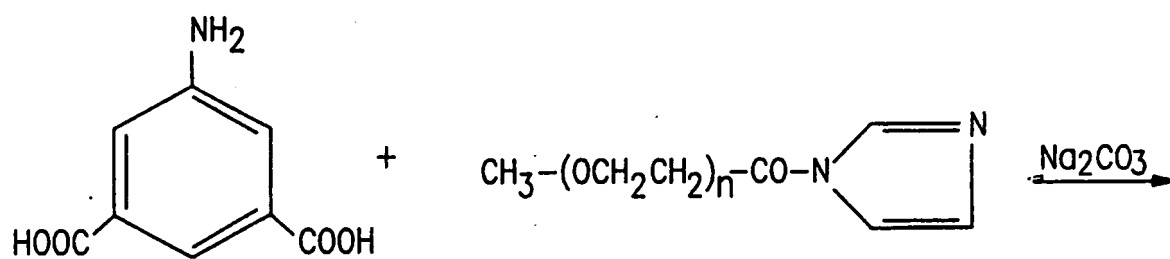


FIG.3

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 93/05595

A. CLASSIFICATION OF SUBJECT MATTER

IPC 5 H01F1/06 G01N33/543 C12N11/00 G01N33/58 C01G1/02
A61K9/127 A61K49/00 C07C69/58 C08G65/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 5 G01N C01G A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	EP,A,0 546 939 (MOLECULAR BIOQUEST, INC.) 16 June 1993 see the whole document ---	1-4,7-12
X	WO,A,91 09678 (OMNI QUEST CORPORATION) 11 July 1991 see the whole document ---	1-4,11, 12
A	EP,A,0 125 995 (ADVANCED MAGNETICS, INC.) 21 November 1984 & US,A,4 628 037 cited in the application ---	
A	WO,A,89 11335 (LIPOSOME TECHNOLOGY, INC.) 30 November 1989 & US,A,4 994 213 cited in the application ---	
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

25 October 1993

Date of mailing of the international search report

03.11.93

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INTERNATIONAL SEARCH REPORT

Int. Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	US,A,4 945 049 (T. HAMAYA ET AL.) 31 July 1990 cited in the application ---	
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